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Name of Candidate: Cynthia Rose
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DISSERTATION AND ABSTRACT APPROVED:

DATE:

Michael Feuerstein 2/27/11
Dr. Michael Feuerstein
DEPARTMENT OF MEDICAL AND CLINICAL PSYCHOLOGY
Committee Chairperson

3/4/11
Dr. Neil E. Grunberg
DEPARTMENT OF MEDICAL AND CLINICAL PSYCHOLOGY
Dissertation Advisor

2/25/11
Dr. Andrew Waters
DEPARTMENT OF MEDICAL AND CLINICAL PSYCHOLOGY
Committee Member

Patricia A Deuster 3/25/11
Dr. Patricia Deuster
DEPARTMENT OF MILITARY AND EMERGENCY MEDICINE
Committee Member

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Cynthia Rose

Cynthia Rose
Medical and Clinical Psychology
Uniformed Services University
03/04/2011

ABSTRACT

Title of Thesis:	The effects of recurrent stress and a music intervention on tumor progression and indices of distress in an MNU-induced mammary cancer in rats
Author:	Cynthia A. Rose, M.S.
Thesis directed by:	Neil E. Grunberg, Ph.D., Professor Department of Medical and Clinical Psychology

The present research examined biological and behavioral effects of recurrent stress, music exposure, and a sound ("noise") control on mammary tumor progression and indices of distress in female rats. All animals were injected with 1-methyl-1 nitrosourea (MNU) to induce mammary tumors. The present research was a 2 x 3 design with stress/no stress and music/noise/no music exposure as the independent variables. The biological variables were: day of first tumor detection, tumor multiplicity, tumor weight, tumor growth, adrenal gland weight, spleen weight, serum corticosterone, and body weight. The behavioral variables were: center time, horizontal activity, and vertical activity, ultrasonic vocalizations, and food consumption.

Major findings in animals that developed tumors include: noise decreased anxiety symptoms; noise increased horizontal activity; sound (music and noise) increased positive affect; noise may be helpful with regard to tumor incidence, tumor growth, and tumor multiplicity in non-stressed animals. The major findings

in animals without tumors include: noise attenuated serum corticosterone when not stressed and sound (music and noise) decreased negative affect.

Limitations to the present experiment include the use of one method of cancer induction and one method of stress manipulation. Additionally, while the music selection was based on current literature, the literature on the effects of music exposure is limited (particularly in animal models) and replication is necessary.

This experiment is valuable in many respects. The “noise” condition was included to serve as a sound control but resulted in several significant findings and, therefore, warrants further investigation. It is clear that the use of a sound control is essential in future research involving music exposure. Future research also should examine the effects of different stressors, different musical selections, and different noises on the effects of stress and tumor progression.

If the experiment is replicated, findings remain consistent, and these results can be extrapolated to the human condition; a sound intervention tailored to an individual (based on tumor status, cancer risk, stress level, depressive symptoms, anxiety symptoms, etc.) may serve as a useful adjunctive treatment. At this time, no clear cut recommendations can be made with regard to the use of a sound intervention.

The effects of recurrent stress and a music intervention on tumor progression and indices of distress in an MNU-induced mammary cancer in rats

by

Cynthia A. Rose

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OVERVIEW

Stress can have negative effects on mental health and physical health (Dozier & Peloso, 2006; Heim & Nemeroff, 2001; Selye, 1956; Baum, Gatchel, & Krantz, 1997). It is not clear whether stress contributes to cancer onset, but there is evidence that stress and breast cancer tumor progression are associated (Ross, 2008). Stress may lead to progression of breast cancer by reducing immune function or by promoting anxiety and depression, which also leads to decreased immune function (Andersen & Wells, 2002). Breast cancer itself is a significant physical stressor and, in addition, people with breast cancer often experience chronic stress related to resulting financial difficulties, social interruptions, and negative side effects from treatments (Andersen, 2003; Andersen, Karlsson, Anderson, & Twefik, 1984).

Stress management techniques in humans can improve mental and physical health by attenuating stress effects (Thornton & Andersen, 2006; Cohen, 2008). Stress management appears to be important in breast cancer patients (Joske, Rao, & Kristjanson, 2006; Carlson & Speca, 2007). Human studies of stress management report potentially beneficial effects to reduce emotional distress and subsequent immune changes that may affect cancer progression (Andersen et al., 2007; Andersen et al., 2008).

Music is a noninvasive relaxation technique that can decrease stress and can be used in combination with traditional pharmacologic treatments and other complementary, alternative, and integrative medicinal approaches (Avers, Mathur, & Kamat, 2007). Therefore, music exposure might reduce stress' impact

on breast cancer. Further, music might serve as an adjunctive therapy for breast cancer patients to reduce stress and, thereby, attenuate any stress-induced exacerbation of tumor progression.

This doctoral research project examined the causal effects of recurrent stress with and without music exposure on indices of distress and tumor progression in female rats with chemically-induced mammary carcinomas. Behavioral measures (including food consumption, anxiety-like behavior, and depressive behavior) and biological measures (including tumor measures, body weight, adrenal gland weights, spleen weights, and a biochemical stress measure) relevant to breast cancer and stress were the focus of this project. The research used an animal (rat) model of mammary cancer and recurrent stress to manipulate stress and to assess detailed behavioral and biological measures (e.g., serum corticosterone, tumor measures). The use of a rat model allows: (1) a true experiment rather than a correlational study; (2) a duration of less than three months, rather than the years it would take in humans; and (3) inclusion of several biological measures that would be difficult to obtain with humans.

The effects of recurrent stress and exposure to a music intervention to attenuate the consequences of stress were examined using a 2 x 3 full factorial design. The independent variables were stress (or no stress), and music exposure (or noise control, or no music/noise) in female rats that were all injected with 1-methyl-1-nitrosourea (MNU), a chemical that induces mammary cancer. A preliminary study was conducted to validate the effectiveness of the stressors (e.g., immobilization restraint combined with various unpredictable stressors) that

was used in this research. The dependent variables were biological (body weight, serum corticosterone, tumor weight, tumor incidence, tumor multiplicity, time until first tumor detection, spleen weight, adrenal glands weight) and behavioral (food consumption, open field locomotor activity marker of distress [anxiety and depression], and ultrasonic vocalizations [to index negative affect]) variables relevant to breast cancer. Tumor measures (time until first tumor detection, tumor weight, tumor growth, tumor multiplicity [i.e., the number of tumors]) were examined to estimate tumor progression. Body weight and food consumption were examined to monitor general health (growth), and because excessive body weight is a risk factor for breast cancer. Locomotor activity and ultrasonic vocalizations were examined to monitor distress symptoms (indices of anxiety and depression).

The specific aims of this research were to determine the effects of: (1) recurrent stress on time until first tumor detection, tumor incidence, tumor growth, and tumor multiplicity; (2) music exposure on stress responses; and (3) music exposure on stress' effects on tumor incidence, growth, and multiplicity.

This doctoral dissertation first reviews the literature on breast cancer, stress, breast cancer and stress, stress management, and music exposure. Next, the rationale for each independent and dependent variable included in this research project is provided. Then, the methods, results, discussion, and conclusions of the project are presented.

Breast Cancer

Breast cancer is a disease characterized by the uncontrolled growth and spread of abnormal tissues that originate in the breast (Williams & Dickerson, 1990). Most carcinogens appear to induce tumors by damaging cellular DNA, leading to a production of abnormal cells (Kiecolt-Glaser, Robles, Heffner, Loving, & Glaser, 2002; Setlow, 1978; ACS, 2008); not fully developed breast cells are particularly sensitive to effects of cancer-causing agents (Clark, Snedeker, & Devine, 1998). The majority of female breast cancer patients are young and older adults (ACS, 2003, 2005). Breast cancer is a carcinoma (tumor originating in the epithelial tissue), but not all carcinomas are the same. Some carcinomas are highly aggressive and quickly metastasize, whereas others grow slowly and may respond better to treatment than fast growing tumors (Weedon-Fekjaer, Lindquist, Vatten, Aalen, & Tretli, 2008; ACS, 2008). In fact, breast cancer can be slow-growing with a long sub-clinical phase that in some women extends more than 18 years (Ginzburg, Wrensch, Rice, Farren, & Spiegal, 2008). The natural history of breast cancer involves the detection of tumors, an increase in tumor size and perhaps number, and possibly then spread to result in fatality. There are no early symptoms of this disease and it is usually recognized with the detection of a tumor. The present research utilized an animal model of mammary cancer, which induces tumors that can be detected by palpation and quantified by numbers and weight following surgical removal.

Histopathology

There are many types of breast cancer. The two major histological categories for breast cancer are *in situ* and invasive carcinoma. *In situ* cancers are usually in the ductal-lobular system and are not as likely to metastasize as invasive carcinomas (Greenfield, 2001). Invasive breast cancer is defined as "tumor cells, which have crossed the basement membrane and have the biologic capacity to metastasize" (Greenfield, 2001, p. 1357).

More than 60% of all breast neoplasias are ductal carcinomas expressing estrogen and progesterone receptors (i.e., estrogen and progesterone receptors are found on breast carcinomas) (Lanari et al., 2009). About 70% of breast cancer tumors express estrogen receptors and are estrogen dependent for growth (Duss et al., 2007; Clark, Snedeker, & Devine, 1998). Estrogens can have a major effect on the development of breast cancer, with about 60% of premenopausal and 75% of postmenopausal breast cancer patients having estrogen-dependent tumors (Duss et al., 2007). In women, the classification of breast carcinomas based on hormonal activity is important because it is used to decide the treatment a patient receives and also is of prognostic importance (Thompson, McGinley, Rothhammer, & Singh, 1998).

Epidemiology

Breast cancer is the second most common cancer among women after skin cancers, accounting for more than 25% of cancers diagnosed in women in the United States (ACS, 2007, 2009). Worldwide, breast cancer is the most frequently diagnosed cancer in women (Garcia, Jemal, Center, Hao, Siegel, &

Thun, 2007). Men, in general, are at low risk for developing breast cancer, although there are occurrences (ACS, 2007; Shah, Robanni, & Shah, 2009). In 2007, there were an estimated 178,480 new cases of invasive breast cancer among women in the U.S. (Garcia, et al., 2007), an estimated 62,030 additional cases of *in situ* breast cancer (has not spread to surrounding tissues) with 80% being ductal carcinomas (confined to breast ducts) (ACS, 2007), and an estimated 465,000 breast cancer deaths in U.S. women (Garcia, et al., 2007). In 2007, about 2,030 cases of breast cancer occurred among men, 1% of all breast cancer cases in the United States (ACS, 2007). In 2008, an estimated 182,460 new cases of invasive breast cancer were diagnosed in U.S. women (ACS, 2008). An estimated 192,370 new cases of invasive breast cancer were diagnosed in the U.S. in 2009, with about 1,910 new cases in men (ACS, 2009). Also, 62,280 new cases of *in situ* breast cancer were diagnosed among U.S. women in 2009, with 85% being ductal carcinoma *in situ*. An estimated 40,610 breast cancer deaths in the U.S. occurred in 2009, making breast cancer the second ranked cause of cancer death (ACS, 2009). An estimated 207,090 new cases of invasive breast cancer and 54,010 new cases of carcinoma *in situ* are expected to occur among U.S. women in 2010 (ACS, 2010). An estimated 39,840 U.S. women are expected to die from breast cancer in 2010. Breast cancer is the second most lethal cancer for women (with lung cancer being first), and the main cause of death for women ages 45 to 55 (Jemal et al., 2006; Ries et al., 2006).

Incidence and mortality rates differ within the United States by ethnicity. According to the American Cancer Society (2007), Caucasian women have the highest incidence rates of breast cancer (132.5 per 100,000), followed by African-American women (118.3 per 100,000), Hispanic women (89.3 per 100,000), Asian Americans/Pacific Islanders (89.0 per 100,000), and American Indians/Alaska Natives (69.8 per 100,000). However, mortality rates do not follow the same ethnic trend as incidence. The death rates are higher, diagnoses at more advanced cancer stage, and trends of more aggressive breast cancer among African-American women, suggesting that genetics, income, and access to medical care also may be important prognostic factors in breast cancer patterns (Bradley, Given, & Roberts, 2002; ACS, 2007). This disparity is partly explained by differences in lifestyle, socioeconomic status, cultural differences in medical-seeking behavior, and access to adequate medical screening and treatment (Bradley, Given, & Roberts, 2002; Smigal et al., 2006).

Prognosis for breast cancer is relatively good as 92% of breast cancers are diagnosed before having metastasized. The five-year survival rate for invasive breast cancer is 98% for localized breast cancer and 83.5% for regional (spread to regional lymph nodes or directly around the primary site) breast cancer. Those with metastasized breast cancer (Stage IV) have a 26.7% five-year survival rate (ACS, 2005; Ries et al., 2006).

Risk Factors

There are many risk factors for breast cancer including age, family history, age at first full term pregnancy, early menarche, late menopause, and breast

density; these factors are not easily modifiable. Other factors associated with increased breast cancer risk include obesity, levels of hormones such as estrogen, increased alcohol consumption, and physical inactivity, which are modifiable (ACS, 2007; Barlow et al., 2006). In fact, a recent study found that women who gained 55 lbs or more after age 18 had a 1.5 greater risk of having breast cancer (Eliassen, Colditz, Rosner, Willett, & Hankinson, 2006; ACS, 2007). Having more fat tissue increases estrogen levels and may increase the likelihood of developing breast cancer (Eliassen et al., 2006; ACS, 2007). Importantly, many of these modifiable factors are correlated with stress, and therefore, stress also may be a possible risk factor that should be investigated.

Impact of Breast Cancer

Each year 200,000 women in the U.S. learn that they have breast cancer (ACS, 2010). Because only about 5-10% cases are thought to be caused by hereditary factors, the diagnosis is often shocking to many women (Ford et al., 1998; ACS, 2008). The resulting emotional distress can affect women's physical and mental health (Lovallo, 2005; APA, 2009). Receiving a diagnosis of breast cancer can be one of the most distressing events a woman encounters (Cruess et al., 2000; APA, 2009).

Stress typically continues even after the initial shock of receiving a breast cancer diagnosis. One third of all oncology patients experience significant distress associated with cancer diagnosis and treatment, with manifestations including: depression, anxiety, fear about mortality or recurrence, fatigue, problems with intimate relationships and social support, destabilization of

finances, facing discrimination from employers and insurance, noncompliance with treatment, cessation of positive health behaviors, and pursuit of negative health behaviors (Varker et al., 2007; APA, 2009; Hansen, Feuerstein, Calvio, & Olsen, 2008). The burdens of cancer can be multiple and chronic (Andersen & Wells, 2002). In fact, stress is a common symptom in women treated for breast cancer (Billhult, Lindholm, Gunnarsson, & Stener-Victorin, 2009), and both cancer and its treatment are generally considered stressful (Kemper, Hamilton, McLean, & Lovato, 2008).

Considerable morbidity persists among survivors of breast cancer, including high levels of psychological stress, anxiety, depression, fear, fatigue, and impaired quality of life (Lengacher, et al., 2009). Diagnosis, treatment, and challenges of survivorship can all potentially increase distress in individuals with breast cancer, which might influence their course of disease, because stress may alter the immune system by genetic changes (mutations in DNA), immune dysregulation, and pro-angiogenic processes (growth of blood vessels and blood supply to tumors)(McGregor & Antoni, 2009). Cancer patients already have poorer immune systems than persons without disease (Andersen, 2003), and the effects of stress on breast cancer patients may further decrease immunity. It is critical to examine the effects of stress on breast cancer detection and progression in individuals with breast cancer because it may be helpful to treat both the cancer and the person with cancer to improve outcome (Stuyck, 2005).

Stress

Stress is a process where stressors challenge an individual, and how an individual interprets these challenges (Baum, Gatchel, & Krantz, 1997; Faraday, 2005; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010). Stress also can be defined as the "process by which environmental demands tax or exceed the adaptive capacity of an organism, resulting in psychological and biological changes that may place a person at risk for disease" (Cohen, Kessler, & Gordon, 1995, p. 3). Stress affects both mental and physical health, such as increasing anxiety, depression, cardiovascular diseases, immune-related diseases, etc. (Dozier & Peloso, 2006; Heim & Nemeroff, 2001; Selye, 1956; Berger, 2009; Baum, Gatchel, & Krantz, 1997; Long, 2010, Perry, 2009; Starosciak, 2010; Hamilton, 2010). Stress is a term that has become common in modern day living (Pant & Ramaswamy, 2009; Long, 2010) and can involve single traumatic events, recurrent events, or chronic conditions. According to the 2008 annual report "Stress in America," 30% of Americans self-reported stress levels as extreme, 50% of Americans reported stress levels that are average, and only 20% of Americans reported stress levels as low (APA, 2008; Segerstrom & Miller, 2004; Long, 2010). In fact, it is estimated that 75-90% of physician visits are the result of stress-related symptoms (Baum, Gatchel, & Krantz, 1997; Burton, 2003; Berger, 2009; Long, 2010). Stress is experienced in a number of ways, through negative emotions, behavioral disruptions, and/or physiological reactions (Grunberg & Singer, 1990; Baum, Gatchel, & Krantz, 1997; Park, Campbell, & Diamond, 2001; Bauer, Perks, Lightman, & Shanks,

2001; Faraday, 2005; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010).

Historical Conceptualizations of Stress

The conceptualizations of stress have changed over the past century with a strong focus first on biological aspects, next on psychological aspects, and then becoming more integrative (Faraday, 2005; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010). Cannon (1929, 1935) viewed stress as a biological process and introduced the fight-or-flight response with the involvement of the sympathetic nervous system when faced with stressors. Hans Selye (1946, 1956, 1973) proposed the General Adaptation Syndrome, where stress was viewed as a nonspecific response of the body to demands for adaptation, primarily involving the Hypothalamic-Pituitary-Adrenal Axis (HPA); exhaustion can occur when an individual no longer has the resources to deal with stressors (Selye, 1946, 1956).

John Mason (1968, 1974, 1975) proposed that an individual's experience of stress depends on the appraisal of a situation, personality/psychological factors, environmental influences, and an integrated multi-hormonal response. Rahe and Arthur (1978) tried to measure stress by examining an individual's level of self-reported stressful experiences. Richard Lazarus and colleagues (Lazarus, 1966; Lazarus & Folkman, 1990) emphasized the contribution of cognitive factors in an individual's response to a stressor. Lazarus and Folkman (1984) recognized the importance of appraisal of a stressful event as well as the appraisal of coping resources. In addition, perceived controllability and

predictability in a person's response to stress were determined to be important factors (Glass & Singer, 1972; Grunberg & Singer, 1990), as well as the fact that stress may have long-lasting effects even after termination of the stressor itself (Glass & Singer, 1972).

Peter Sterling and Joseph Eyer (1984) introduced allostasis, or the process of maintaining stability through flexibility (Long, 2010; Berger, 2009). Bruce McEwen (1998, 2004) added to allostasis by suggesting that imbalances with repeated stressors can lead to allostatic overload, where processes are no longer able to lead to adaptation (McEwen, 1998, 2004). It is clear that biological, psychological, and environmental variables are all relevant to stress responses (Faraday, 2005; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010).

Biological Responses to Stress

The stress response is a complex stream of events that occurs after encountering a stressor (Charney, 2004; Guyton & Hall, 2000; Conti, 2000). The stress response includes activation of the hypothalamic-pituitary-adrenal axis (HPA) and the sympathetic nervous system (SNS) (Guyton & Hall, 2000). In the HPA system the hypothalamus releases corticotropin releasing factor (CRF). CRF stimulates the pituitary release of adrenocorticotropin hormone (ACTH), and ACTH stimulates the adrenal glands to release cortisol (Charney, 2004; Guyton & Hall, 2000). Cortisol mobilizes glucose from energy stores; increases arousal, vigilance, and attention-enhancing memory formation; and inhibits immune system functioning (Guyton & Hall, 2004). When the SNS is activated, there is a

release of catecholamines, including norepinephrine and epinephrine (Baum, Grunberg, & Singer, 1982; Babisch, 2003), as well as an increase in heart rate, blood pressure, respiratory rate, blood flow to large muscle groups and the brain, an increase in glucose release, pupil dilation, and a decrease in blood flow to the digestive tract and reproductive organs (Baum, Gatchel, & Krantz 1997; Lovallo, 1997). Therefore, the stress hormones epinephrine, norepinephrine, and cortisol can serve as stress indicators (Babisch, 2003).

Stress can alter the immune system, making individuals more vulnerable to colds and flu, fatigue, and infections (Bock & Weeks, 2002). In response to infections, the immune system produces three key substances that cause inflammation: interleukin 1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor (TNF) (Bock & Weeks, 2002). These substances can also cause the release of CRF, which in turn can promote the release of ACTH (released by the pituitary gland in response to stress) and cortisol (Conti, 2000; Bock & Weeks, 2002). Cortisol and other compounds then suppress the release of IL-1, IL-6, and TNF switching off the inflammatory response (Bock & Weeks, 2002). Ideally, stress hormones depress the immune response that has run its course, but this continuous activation of the HPA axis may lead to a decreased ability to release interleukins and fight infection (Bock & Weeks, 2002).

Psychological stress might alter immune function by direct interaction with lymphatic tissue and by stress-elicited release of hormones from the brain that alter the functions of immunologically-active cells (Cohen & Rabin, 1998). Stress can produce biological responses that range from activation of the HPA axis to

altering the physiology of internal organs and organ systems (Kvetnansky, Weise, & Kopin, 1970; Keim and Sigg, 1976; Martijena, Cavlo, Vosolin, & Monlina, 1997; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992; Pham, Soderstrom, Henriksson, & Mohammad, 1997; Bielajew, Konkle, & Merali, 2002; Bauer, Perks, Lightman, & Shanks, 2001; Elliott, Faraday, & Grunberg, 2003). For example, stress in animals attenuates immune functioning, decreases the thymus and other lymphatic organs, and elicits actions of adrenocortical hormones (Justice, 1985). It is important to understand the impact of stress on immune function, because immune impairment is critical for cancer progression. As a gross indicator of immune function, the present experiment examined spleen weights because it is an important lymphatic organ.

Although the present experiment did not measure specific immune markers, cortisol was measured, and therefore, it is important to understand how cortisol and HPA axis activation can impact immune function and ultimately cancer progression. Glucocorticoids, such as cortisol, are believed to have strong effects on the immune system, and catecholamines, CRF, and opioids can play a role as well (Thornton & Andersen, 2006). Lymphocytes and macrophages have receptors that are responsive to high levels of circulating glucocorticoids (Thornton & Andersen, 2006). Glucocorticoids can directly suppress the action of t-lymphocytes and macrophages and may affect cell circulation (Thornton & Andersen, 2006). This suppression, in turn, affects the production and release of cytokines, such as interleukin 2 (IL-2) and interferon γ (IFN γ) that can exert influence on immune function (Thornton & Andersen,

2006). Lymphocytes and macrophages also have catecholamine receptors, and catecholamine and glucocorticoid imbalance could increase an individual's risk for infection and disease by altering cytokine secretions and impairing the function of natural killer (NK) cells (Thornton & Andersen, 2006). Cytokines such as INF γ and IL-2 can enhance NK cell and lymphocyte-activated killer cell cytotoxicity (Kiecolt-Glaser et al., 2002; Herberman & Ortaldo, 1981), but stress decreases IFN γ and IL-2 synthesis (Kiecolt-Glaser et al., 2002; Dobbin et al., 1991; Glaser et al., 1987; Glaser et al., 1986). In summary, the biologic response to prolonged stress could make the body more susceptible to disease, by switching off disease-fighting white blood cells (Bock & Weeks, 2002), and decreasing NK cell lysis, t-cell numbers and function (Andersen, 2001). These disease-fighting immune responses are critical to control cancer progression.

Meta-analytic reviews report consistent immune changes in the presence of psychological stressors, such as reductions in NK cell cytotoxicity, t-cell proliferation, and antibody response (Thornton & Andersen, 2006). Chronic stress reduces the ability of the immune system to handle new challenges. With greater distress, NK and t-cell function decrease and interleukin 6, which is a predictor of future disability, increases (Thornton & Andersen, 2006). NK cells have receptors for neuropeptides, opioids, prolactin, and other hormone regulating interactions between the brain and immune system. NK cells are highly responsive to changes in the serum levels of these hormones, and stress often involves all of these hormones (Whiteside, Baum, & Herberman, 2000). Effects of stress on the immune response can be reliably monitored by following

changes in NK cell activity. Acute stress responses are associated with SNS stimulated rapid release of catecholamines and NK cells, whereas chronic stress is associated with suppression of NK cells because of slow-acting hormonal systems. The HPA axis typically has suppressive, non-reversible influences on the immune system, suggesting that NK cells may be a useful immune marker of stress (Whiteside, Baum, & Herberman, 2000).

The HPA axis communicates with several regions of the brain, altering mood, anxiety, pain, and appetite (Steckler, Kalin, & Reul, 2005; Bock & Weeks, 2002). The HPA axis also interacts with other glandular systems, among them reproductive hormones, growth hormones, and thyroid hormones (Bock & Weeks, 2002). The stress response turns off hormonal systems regulating growth, metabolism, and immunity (Bock & Weeks, 2002). In the short term this response is helpful. However, the HPA axis may be permanently altered as a result of extreme stress. Stress suppresses the reproductive system when CRF prevents the release of gonadotropin releasing hormone, which signals the release of hormones that direct reproduction and sexual behavior. Cortisol and related glucocorticoid hormones also release luteinizing hormone, which inhibits testes and ovaries directly, hindering production of the male and female sex hormones testosterone, estrogen, and progesterone. The female hormone estrogen exerts partial control of the gene that stimulates CRF production, which is why women have increased cortisol levels and may be the reason why women experience more depression and anxiety disorders in general than men (Bock & Weeks, 2002). The relationship between stress and estrogen is unclear

(Charney, 2004). Females consistently show greater physiological response to both acute and chronic stressors, which many investigators attribute to sex hormone differences (Charney, 2004). Stress effects on estrogen are particularly important in breast cancer progression because breast carcinomas may have active estrogen receptors.

Psychological Effects of Stress

Stress also has detrimental psychological effects that impact immune function. Mental health consequences of stress include depression and anxiety. Stress can lead to depression and anxiety through glucocorticoids and/or overactivity of the SNS (Baum, Cohen, & Hall, 1993). Americans list irritability, anger, fatigue, anxiety, sadness, lack of interest, motivation, and energy as common psychological symptoms of stress (APA, 2008). Stressful events can trigger cognitive and affective responses which, in turn, can induce SNS and endocrine changes. These changes can impair immune functioning which could influence cancer progression (Thornton & Andersen, 2006). Affect changes in response to stressors can provide measures of well-being that may capture sub-clinical symptoms of psychological impairment.

One of the major disorders characteristic of an overactive HPA axis is depression (Bock & Weeks, 2002). Both acute and chronic stressors may precipitate the occurrence of Major Depressive Episode (Hammen, 2005). There also is a positive relationship between mood disturbances and life stress for patients (Andersen & Wells, 2002). Stressful life events can often lead to depressive symptoms (Caspi et al., 2003). Depressed patients may have greater

impairment across multiple physiological systems because of alterations in central nervous system regulation, the central serotonergic system, and through descending pathways, and this impairment may alter subjective, immune, and autonomic function (Giese-Davis et al., 2006). People with depression may have a blunted ability to counter-regulate or adapt to negative feedback and increases in cortisol (Bock & Weeks, 2002). Insomnia, a common symptom of depression, also increases cortisol (Bock & Weeks, 2002). Depressed patients may have less sensitivity to the antinflammatory aspects of cortisol and cytokines leading to increased heart rate and higher basal glucocorticoid levels (Giese-Davis et al., 2006). Depressed individuals often show a reduced NK cell lysis and significantly poorer repair of damaged DNA (Ferrer, 2007; Kiecolt-Glaser et al., 2002). The present research examined how stress and tumor progression are related to negative affect.

Chronic stress can produce constant anxiety (Bock & Weeks, 2002). Both acute and chronic stressors may precipitate the occurrence of anxiety disorders (Shearer, 2007). The animal literature suggests that stress often leads to anxiety behaviors. For example, in response to inescapable foot shock or immobilization, rodents decrease overall activity and increase defecation in an open field arena (Gamallo, Villanua, Trancho, & Fraile, 1988; Van Dijken, Mos, Van der Heyden, & Tilders, 1992; Faraday, 2002). Predator stress impairs habituation to a novel environment by increasing activity within open field (Park, Campbell, & Diamond, 2001). Exposure to inescapable shock decreases time in open arms in the elevated plus maze (EPM) (Steenbergen, Heinsbroek, Van

Hest, & Van de Poll, 1990; Martijena et al., 1997; Kalinichev, Easterling, Plotsky, & Holtzman, 2002). Stress can interrupt learning and memory, increase anxiety-like behaviors, affect cognitive performance, interrupt attentional tasks measured by prepulse inhibition of the acoustic startle reflex (Acri, 1992; Faraday, 2002), and decrease spatial learning and memory in the radial arm maze (Park, Campbell, & Diamond, 2001). The present research examined how stress and tumor progression relate to anxiety-like and depressive-like behaviors.

Behavioral Effects of Stress

There also are behavioral changes in response to stress (Cohen & Rabin, 1998). Stress can promote behavioral changes, including less healthy behaviors, such as getting less sleep, less exercise, increasing alcohol intake, increasing tobacco smoking, increasing caloric intake, and eating more fat and sugar (Thornton & Andersen, 2006; Andersen, 2003; Bock & Weeks, 2002). Stress' impact on appetite may lead to changes in feeding and body weight (Acri, 1992; Faraday, 2002). Increased levels of cortisol can increase appetite and lead to weight gain (Bock & Weeks, 2002). There is some evidence that stress-induced changes in appetite may lead to obesity (Greeno & Wing, 1994), especially in women (Grunberg & Straub, 1992). The animal literature also supports that stress affects feeding behaviors. For example, rats crowded, experiencing changes in housing environments, exposed to electric shock, and exposed to restraint stress decrease food consumption (Brown & Grunberg, 1995; O'Connor & Eikelboom, 2000; Rickards, Job, & Boakes, 1997; Marti, Marti, & Armario, 1994; Zylan & Brown, 1996). In contrast, exposure to repeated cold stress as

well as noise stressors increase food consumption (Kawanishi, Fukuda, Tamura, Nishijo, & Ono, 1997; Krebs, Macht, Weyers, Weyers, & Jankers, 1996). The present research examined how stress affects feeding behavior because excessive body weight is a relevant risk factor in individuals with breast cancer.

Stress and Breast Cancer

Psychological Effects of Stress and Breast Cancer

Stress is an important predictor of diseases that occur when there is an immune dysregulation, such as acquired immune deficiency syndrome and influenzas (Cohen & Rabin, 1998); stress is an important factor in individuals with cancer as well. While the link between stress and breast cancer susceptibility is not yet understood because of limitations of self-report and difficulties assessing cancer occurrence (Segerstrom & Miller, 2004), the association between stress and breast cancer tumor progression is strong (Ross, 2008).

Stress appears to be a common factor in the lives of people with breast cancer. Often individuals with breast cancer experience anxiety related to fear of death, changes in lifestyle, an increase in stressors, and altered body image (Eschiti, 2008). Another stressor common to individuals with breast cancer is relationship difficulties, and marital distress in breast cancer patients has been associated with slow recovery, poor outcomes, and signs and symptoms of illness and treatment side effects than those not experiencing marital distress (Yang & Schuler, 2009). Depressive symptoms are significantly more likely in distressed breast cancer patients (Yang & Schuler, 2009). Breast cancer patients who reported stressful life events have a two-fold risk of breast cancer

recurrence (Palesh, Butler, Koopman, Giese-Davis, Carlson, & Spiegel, 2007), and prolonged stress exposure may interfere with the body's ability to fight off cancer progression (Palesh et al., 2007). In fact, stress predicts poor survival and high cancer mortality among individuals with breast cancer than individuals that are not experiencing stress (Andersen et al., 2008), and cancer-related stress is correlated with impaired immunity in patients with invasive breast cancer (Varker et al., 2007).

The most salient emotions that occur with an initial or recurrent diagnosis of cancer are fear, anxiety, and depression (Andersen & Wells, 2002). Anxiety is common for individuals diagnosed with a medical condition, and depression is common for conditions where there is threat to life (Andersen & Wells, 2002). Stress appears to promote anxiety and depressive symptoms in people with breast cancer. The diagnosis of breast cancer often leads to high levels of stress (Yang, Brothers, & Andersen, 2008). Levels of distress, depression, and anxiety are substantially high among patients with breast cancer (Spiegel, 1997). Severe, acute stress often occurs at the time of cancer diagnosis, and cancer patients often report chronic stress that contributes to emotional distress, life disruptions, and lower quality of life (Andersen, 2003). Chronic stressors that typically occur with a cancer diagnosis are subsequent financial, insurance coverage, and employment difficulties, with as many as 20% of cancer patients reporting chronic economic difficulties (Andersen, 2003). One third of all oncology patients experience significant distress associated with cancer diagnosis and treatment (Varker et al., 2007). In a study with cancer patients, as

time of cancer treatment neared, subjective and physiologic indicators of anxiety and distress significantly increased and remained elevated post-treatment and patients continued to respond with anxiety and distress in subsequent treatments (Andersen, Karlsson, Anderson, & Twefik, 1984). Cancer diagnoses and treatments are negative events (Andersen, Kiecolt-Glaser, & Glaser, 1994) that contribute to severe emotional distress, and chronic stressors can occur with cancer-causing immune changes associated with these stressors (Andersen, Kiecolt-Glaser, & Glaser, 1994).

Specific Biological Effects of Stress and Breast Cancer Progression

Stress increases the body's production of the stress hormones cortisol (or corticosterone in rats), epinephrine, and norepinephrine, which may dysregulate the immune system and decrease cell-mediated immunity, which is specifically relevant for cancer patients because cancerous cells may have a better chance of surviving and spreading (Stuyck, 2005). An increase in norepinephrine can stimulate tumor cells to produce two compounds, matrix metalloproteinases MMP-2 and MMP-9, which break down the tissue around the tumor cells and allow the tumor cells to more easily move into the bloodstream (Glaser, 2006; Lutgendorf et al., 2003). Catecholamines and cortisol also can stimulate the tumor cells to release VEGF, which can aid in the growth of new blood vessels that feed cancer cells, hastening the growth and spread of disease (Glaser, 2006; Lutgendorf et al., 2003; 113). MMP-2 and MMP-9 contribute to the aggressiveness of tumors (Glaser, 2006). Chronic behavioral stress results in higher levels of tissue catecholamines, greater tumor burden, and more invasive

growth of ovarian carcinoma in a mouse model (Thaker et al., 2006). Epinephrine can make breast cancer cells resistant to cell death because the protein BAD (BCL-2 antagonist of cell death), which causes cell death, becomes inactive when cancer cells are exposed to epinephrine (Richardson, 2007).

Stress can alter immune system functioning, such as activity of NK cells, and the activity of NK cells is related to breast cancer progression. However, the link between stress and breast cancer is not clear (Ross, 2008). Cancer-related psychological stress is associated with reduced NK cell lysis, but the exact mechanisms are unknown (Varker et al., 2007). NK cells play a role in tumor growth inhibition and surveillance against newly developing primary tumors (Levy, 1983), and cortisol decreases NK cells. Stressors are associated with decreased cytotoxic T-cells and NK cell activities that affect processes such as immune surveillance of tumors and development and accumulation of mutations and genomic instability (Reiche, Nunes, & Morimoto, 2004). NK cells resist the progression and metastatic spread of tumors once they have developed (Richardson, 2007; Azar, 1999; Kiecolt-Glaser et al., 2002). NK cell function negatively predicts number of tumor nodes, tumor size, recurrence rate, lymphocyte proliferation, and rate of recurrence and survival (Cohen, 2008; Azar, 1999). Decrements in NK cell counts are an important predictor in advanced breast cancer survival (Rao et al., 2007), and high NK cell activity is a strong predictor of disease free survival (Azar, 1999). Increased stress in breast cancer patients decreases NK cells, and NK cells have important functions with regard to cancer because they are capable of detecting and killing cancer cells

(Anderson, 2003). Stress decreases NK cell activity in rats and can cause a two to five-fold increase in certain types of tumors and promote metastasis (Azar, 1999). In a human study of breast cancer patients, patients with the highest reported stress had lower levels of NK cell lysis (Andersen, 1998).

Stress also damages DNA, which is an important factor in breast cancer. Stress leads to poorer repair of damaged cellular DNA and alters apoptosis (Kiecolt-Glaser et al., 2002). Stress affects apoptosis, a process of genetically-programmed alterations in cell structure that leads to failure of proliferation and differentiation and eventual cell death (Kiecolt-Glaser, 2002). Glucocorticoids inhibit apoptosis of human mammary cells (Ross, 2008). Stress impedes cells' ability to repair DNA damage, and failure to repair DNA damage is one of the first stages of cancer development and is critical in cancer progression (Azar, 1999). Stress can cause DNA damage, and animal research indicates that stress is associated with tumor progression (Cohen, 2008).

Cancers that are etiologically related to hormonal stimuli may be most susceptible to influence of stress, as would be expected with breast cancer because the majority of breast carcinomas are hormone-dependent (Andersen, Kiecolt-Glaser, & Glaser, 1994). Development and progression of breast cancer are directly related to the effects of the female hormone estrogen through pathways mediating cell survival, cell proliferation, and response to stress (Osborne, Schiff, Fuqua, & Shou, 2001). Stressful stimuli also can alter neuroendocrine activity resulting in increased secretory rates of endocrine stimulatory growth factors (i.e., prolactin, estrogen, corticosterone) which could

directly impact tumor cell development (Welsch, 1985). The impairment in estrogen synthesis may explain incidence of breast cancer (Nielsen, Zhang, Kristensen, Netterstrom, Schnohr, & Gronbaek, 2005). Estrogen is a strong risk factor for breast cancer, and stress may increase estrogen, increasing breast cancer risk and progression (ACS, 2007). In addition, in 70% of breast cancers patients' cortisol circadian rhythms are disrupted, and these aberrations have been linked to the stress of cancer itself and the psychological stress of the disease (Turner-Cobb, Sephton, Koopman, Blake-Mortimer, & Spiegel, 2000). Cortisol inhibits glucose uptake by normal cells, whereas tumor cells may become resistant to this effect and therefore have a metabolic advantage (Turner-Cobb et al., 2000), and aberrations in cortisol rhythms may increase cancerous cells' metabolic advantage. With regard to breast cancer in women, stress may not only increase tumor progression through its action on estrogen, but it also may speed up tumor progression by disrupting cortisol rhythms. Based on the evidence that stress alters biological parameters that are important to immune function and may be particularly detrimental to cancers that are etiologically related to hormonal stimuli, the present research examined the effects of stress in a breast cancer animal model.

Biobehavioral Plausibility

Emotional reactions of newly diagnosed cancer patients are severe with reports of clinically significant depressive and anxiety symptoms being common (Fowler, Carpenter, Gupta, Golden-Kreutz, & Andersen, 2004; Simonelli, Fowler, Maxwell, & Andersen, 2008). Twenty percent of cancer patients are so

distressed that they meet criteria for a psychiatric diagnosis, such as major depressive disorder, and the diagnosis is often attributed to a cancer diagnosis (Fowler et al., 2004). Twenty to sixty percent of cancer patients experience depressive symptoms that affect treatment compliance, and missing as few as 15% of chemotherapy appointments results in significantly poorer health outcomes (APA, 2009; Fanguard & Schnoll, 2002). People with depressed mood had a cancer death rate 2.3 times higher than those people without depressed mood, and helplessness and hopelessness are believed by many researchers to decrease survival in people with cancer (Fox, 1995). Stress-induced anxiety and depression have been associated with increased mortality in cancer patients (Nunez et al., 2002; Giese-Davis et al., 2007). Depression is associated with the decreased cytotoxic T-cell and NK cell activities that affect processes such as immune surveillance of tumors, and alter the development and accumulation of mutations and genomic instability (Reiche, Nunes, & Morimoto, 2004). Helplessness also plays a role in cancer progression because it is associated with decreased NK activity and cancer progression (Levy, Herberman, Maluish, Schlien, & Lippman, 1985). Animal studies have reported that helpless animals have depleted norepinephrine in the brain, increased release of corticosterone, and subsequent immune suppression of the NK cells (Levy et al., 1985) as compared to non-helpless. In addition, stressed mice with depressive symptoms had higher vascular endothelial growth factor (VEGF) (stimulates growth of new blood vessels), more blood vessels (needed for cancer to be supplied with nutrients to grow and spread), and higher levels of catecholamines in the

periphery than non-stressed mice (Ross, 2008). Exposure to stressors such as uncontrollable aversive events appears to increase anxiety and depressive symptoms (Maeir & Watkins, 2005) in people with breast cancer and these symptoms appear to lead to further declines in immune function. Therefore, the present research examined behaviors related to anxiety and depression as a result of exposure to uncontrollable stress and evaluated how these behaviors related to tumor progression after injection of a chemical carcinogen in a rat model of breast cancer.

Animal Literature on Stress and Breast Cancer

The animal literature on the role of stress in breast cancer progression is compelling. For example, restraint stress increases mice MCa (3-methylcholanthrene chemical induction) mammary carcinoma metastasis and reduces the beneficial effects of cyclophosphamide (a chemotherapy agent) (Giraldi, Zorzet, Perissin, & Rapozzi, 2000). Adrenoceptor agonists, chemicals that increase the stress response, enhanced proliferation of the mouse mammary tumor cell line MC4-L5 and stimulated tumor growth of progestin-dependent tumors (Bruzzone et al., 2008). It also has been reported that isolated female rats developed mammary tumors at a much higher rate than socially housed rats (Ross, 2008). Female rats with mammary adenocarcinoma had increased tumor burden with restraint stress and also had decreased leukocyte production (Steplewaki, Vogel, Ehya, Poropatich, & Smith, 1985). In a 7,12-Dimethylbenz(a)anthracene (DMBA) chemically-induced mammary cancer in rats, exercise-induced stress and forced swimming enhanced adrenaline,

prolactin concentrations, and enhanced mammary carcinogenesis in DMBA rats (Saez, Barriga, Garcia, Rodriguez, & Ortega, 2007). In a study where animals were stressed before DMBA carcinogen administration, these rats showed higher frequencies of damage to DNA than just the administration of DMBA alone, and later exposure to stress enhanced DMBA-induced DNA damage (Muqbil, Azmi, & Banu, 2006). Chronic restraint stress on DMBA-induced rats developed a greater number of tumors earlier and had decreased body weight compared to unstressed rats (Tejwani, Gudehithlu, Hanissian, Gienapp, Whitacre, & Malarkey, 1991). Exposure to magnetic field stress increased incidence of tumor in DMBA-induced and 1-Methyl-1-Nitrosourea (MNU), chemically-induced mammary cancer in rats (IARC, 2002). Exercise-induced stress also can enhance the tumorigenic response in MNU rats (Thompson, 1994). However, effects of recurrent psychological stress have not been examined in the MNU model of mammary cancer in rats.

Summary

Chronic and acute stressors appear to promote tumor growth in individuals with breast cancer (Azar, 1999). Stress may reduce host resistance in tumor growth (Palesh et al., 2007). There is extensive evidence that stress suppresses cell-mediated immunity, a component of the immune system involved in tumor surveillance (Stuyck, 2005). In addition, anecdotal evidence such as self-reported stressful events and clinical observations have suggested that exposure to psychosocial stress affect disease outcomes in immune-related disorders such as tumors (Cohen, 2008), and may be particularly important in

hormone-dependent tumors including most breast cancers. It also may be critical not only to treat the tumors in breast cancer patients, but also to treat the stress that often accompanies this disease. The present research examined the effects of a psychological stressor on tumor progression in an MNU model of mammary cancer in female rats. The present research also examined the effects of listening to music as a possible stress management technique to alter effects of stress on tumor progression.

Stress Management

Stress management was developed and premised on a mind-body interaction conceptualization of stress. Stress management is based on the idea that stress is not solely the result of a direct response to a stressor, but also results from appraisal of resources and ability to cope with the stressor (Lehrer, Woolfolk, & Sime, 2007). Stress management suggests that interventions can mediate the stress response and that stress responses are able to change, allowing stress to be controllable (Lehrer, Woolfolk, & Sime, 2007). Stress management can take the form of a behavioral, cognitive, or pharmacological intervention (Lehrer, Woolfolk, & Sime, 2007; Long, 2010). Some behavioral stress management techniques are deep-breathing, muscle relaxation, exercise, and music therapy (Lehrer, Woolfolk, & Sime, 2007). Cognitive stress management techniques include problem-focused or emotion-focused strategies, and pharmacological techniques include the use of benzodiazepines, antidepressants, antihistamines, d-cycloserine, beta adrenergic receptor antagonists, anticonvulsives, and buspirone (Papp, 2007). Cognitive techniques

may not be appropriate when interacting with individuals who have brain injury, mental retardation, and/or thought disorders (Lehrer, Woolfolk, & Sime, 2007; Long, 2010). Pharmacological strategies have multiple side effects including diet restrictions, weight gain, and sexual dysfunction (Papp, 2007). Behavioral treatments offer a broader range of options with fewer restrictions and minimal risks, perhaps making it preferable to patients with medical conditions such as cancer because they may already be experiencing treatment side effects as well as cognitive disruptions.

Mind-body programs and other psychosocial interventions are a useful adjunct to conventional medical interventions (Cohen, 2008). Psychological interventions have sought to improve immune function by reducing subjective experiences of stress (Thornton & Andersen, 2006). Relaxation interventions appear to have reliable effects on immune function with immunological benefits that correspond to psychological benefits (Thornton & Andersen, 2006). Stress management interventions have been reported to reduce distress, reduce pain and discomfort, reduce sympathetic arousal, buffer immune suppression, improve cognitive function, decrease fatigue, improve quality of life, enhance survival, reduce health care costs (Cohen, 2008), improve emotional adjustment and quality of life (Andersen, Shelby, & Golden-Kreutz, 2007), improve dietary behaviors, and decrease anxiety, depressive symptoms, perceived stress, salivary cortisol (Raghavendra et al., 2009), and symptoms and signs of disease (Andersen, Shelby, & Golden-Kreutz, 2007).

Complementary and alternative medicine (CAM), which include variations of stress management techniques, may be useful to treat cancer (Joske, Rao, & Kristjanson, 2006). CAM use among cancer patients is reported to be between 31.4 and 69% (Joske, Rao, & Kristjanson, 2006). Between 48 and 70% of women with breast cancer report using CAM (Eschiti, 2008). A cross-sectional, retrospective study reported that women with breast cancer experience psychological distress, including anxiety and depression, and many women use CAM to relieve such distress (Eschiti, 2008). Sometimes mainstream treatments such as surgery, chemotherapy, and radiation therapy, for breast cancer can have disturbing side effects that are considered too undesirable to tolerate (ACS, 2008).

Psychological interventions can reduce emotional distress for breast cancer patients and may improve health outcomes and immune function (Andersen et al., 2007). These interventions may prevent immune changes that are secondary to stress hormones and that may promote cancer growth or metastasis (Andersen et al., 2008). Stress management has been reported to decrease depression in breast cancer patients (Antoni et al., 2001). Psychological interventions have been reported to lower stress, decrease the chemotherapy dosages required to be effective, promote more positive health behaviors and fewer negative ones, and increase immune responses (Andersen et al., 2004). Interventions designed to reduce cancer-related stress and enhance mood also could influence biological responses (Andersen, 2001). There is evidence that stress management interventions improve t-cell

blastogenesis (Andersen et al., 2007), decrease the deterioration of NK cell activity, lower heart rate and systolic blood pressure, lower breast cancer specific anxiety and general anxiety, lower cortisol, increase lymphocyte proliferation, and increase NK cell counts in patients with breast cancer (Bilhut et al., 2009; Antoni et al., 2009; McGregor & Antoni, 2009; Antoni et al., 1991). Because stress management appears to be beneficial to decrease stress and possibly cancer progression, the present research examined the effects of a stress management intervention, music exposure, on stress responses (biological and behavioral) and tumor progression.

Music

Music can be used for stress management. Listening to and producing music has played an important role to promote human well-being since ancient times (Abrams, 2001; Kemper et al., 2008; Lehrer, Woolfolk, & Sime, 2007). In fact, music and medicine have been closely associated for centuries (Munro & Mount, 1978), and music has been reported to lower blood pressure, decrease heart rate, and create a sense of well-being (Erken, Bor-Kucukatay, Erken, Kursunluoglu, & Genc, 2008). Music, a universal language with many purposes, has been used in the health care setting to reduce stress and anxiety (Nunez et al., 2002). Music is an appealing, noninvasive relaxation technique to lower stress and can safely be used in combination with traditional pharmacologic treatments and other complementary and alternative medicines (Avers, Mathur, & Kamat, 2007). Music is a nonpharmacological intervention for a diversity of challenges such as physical, psychosocial, and spiritual challenges, and it can

alter cognitive, affective, and sensory processes (Conti, 2000). The use of music as a therapeutic intervention is simple and inexpensive, and takes little time to implement, yet the mechanism of how music produces these effects is still not clear (Erken et al., 2008).

Music has been used to decrease anxiety and discomfort, is well tolerated, reduces patient's perception of unpleasantness, and lessens the possibility of refusal of repeated procedures (Smolen, Topp, & Singer, 2002). Music has been used effectively to reduce stress in procedures such as childbirth, bronchoscopy, medical/dental treatments, and during acute myocardial infarctions (Smolen, Topp, & Singer, 2002). Characteristics of anxiolytic music include simple repetitive rhythm, predictable dynamics, low pitch, slow tempo, consonance of harmony, lack of percussion, and lack of vocals (Watkins, 1997). These characteristics of music lower physiological responses associated with stress, such as blood pressure, heart rate, and ACTH release, whereas music without these characteristics like rock music increases these stress responses (Watkins, 1997). The beneficial effects of music may be greater with repeated use (Watkins, 1997). However, there is limited information on nonpharmacological treatments such as music (Nunez et al., 2002), which is why the present research examined the effects of a music exposure, as a form of stress management, on stress responses and tumor progression.

Music is reported to improve measures of anxiety, fear, fatigue, relaxation, and diastolic blood pressure and encourages emergence of positive feelings and physical activity (Aitini et al., 2007; Ferrer, 2007; Abrams, 2001; Knight &

Rickard, 2001; Munro & Mount, 1978; Avants, Margolin, Salovey, 1991). Salivary cortisol increased significantly less in people listening to music during a colonscopic exam, and music decreased heart rate, blood pressure, and self-reported anxiety (West, 2004; Smolen, Topp, & Singer, 2002; Uedo et al., 2004). Joske, Rao, and Kristjanson (2006) reported increases in salivary Immunoglobulin A after music. In another study, participants who listened to music had enhanced mood, lower gene expression levels of the stress-induced cytokine interleukin 10, and higher NK cell activity compared to participants that did not listen to music (Rao et al., 2007). Abrams (2001) reported that listening to music increased neutrophil and lymphocyte cell counts and decreased urinary levels of corticosteroids. In a meta-analysis based on 22 quantitative studies, music significantly decreased arousal from stress, skin conductance, blood pressure, and heart rate (Allen & Blascovich, 1994). When listened to regularly, music decreases cortisol and boosts immunity (Akombo, 2007).

Music may provide stress relief by decreasing muscle tension (Reynolds, 1984; Munro & Mount, 1978). Music may mediate changes in blood pressure, heart rate, and anxiety levels by affecting the release of CRF from the hypothalamus or the release of norepinephrine from the locus coeruleus and SNS (Erken et al., 2008). The auditory system automatically activates the reticular activating system, can evoke autonomic-neuroendocrine responses, and may be an explanation of how music alters the stress response (Babisch, 2003). Mechanisms involved in the reduction of anxiety and stress by music could be direct inhibition of the expression of some stress-induced genes or the alteration

of some of the opiate and cytokine processes in the listeners, such as decreased secretion of IL-6 (Bozcuk et al., 2006). In addition, music may reduce anxiety by altering thoughts, emotions, or moods and by inducing relaxation (Munro & Mount, 1978; Kwekkeboom, 2003). Music reduces stress-induced hyperactivity of the HPA axis involving ACTH and corticosteroid secretions, and alters norepinephrine, epinephrine, growth hormone (GH), prolactin (PRL), and beta endorphin secretion (Nunez et al., 2002; Lindblad, Hogmark, & Theorell, 2007). When music was in a major key, salivary cortisol was reduced and there was activation in the upper temporal cortex where emotional processing occurs and may be related to stress reduction (Suda, Morimoto, Obata, Koizumi, & Maki, 2008). In addition, emotional responses in the hypothalamic region, which activates pituitary and other endocrine processes, are affected by music (Abrams, 2001). Music may have relaxing effects through the mechanism of entrainment, or the natural tendency to synchronize with other events. Specific elements of music can enhance the physiological system, especially heart rate, vascular dilation, oxygenation, and autonomic activity, which may synchronize with musical tempo, meter, melodic phrasing, and other musical elements (Abrams, 2001). Some music appears to be as effective as pharmacological interventions, such as benzodiazepines and serotonin antagonists, to attenuate stress-induced immunosuppression (Nunez et al., 2002). The present research examined effects of music on biological and psychological responses to stress.

Not all studies that examined the effects of music have found music to reduce stress responses. One human study reported that neither stress

hormone nor beta endorphin was influenced by listening to music (von Allmen, Escher, Wasem, & Fischer, 2004). Another human study reported that listening to music was associated with increased norepinephrine and cortisol (Babisch, 2003). These contradictory findings may reflect the type of music that was used in each study. Unfortunately, many reports regarding music do not provide sufficient detail regarding the music that was used. Most studies that have found anxiolytic effects of listening to music have used self-selected music or classical music.

Self-selected music has been reported to lower anxiety and treatment-related distress, with greater exposure to music producing greater declines in distress (Clark et al., 2006). In addition, listening to self-selected music may lead to positive emotions created by enhanced parasympathetic activity, reduced cortisol, and boosted immunity (Akombo, 2007). Both classical and self-selected relaxing music increase perceptions of relaxation to a greater degree than does listening to hard rock music (Erken et al., 2008). Labbe, Schmidt, Babin, and Pharr (2007) reported that humans listening to classical or self-selected music had decreased negative emotion and physiological arousal after a stressful test. Classical music has been linked to improvement in mood (Erken et al., 2008), a significant increase in secretory Immunoglobulin A in human saliva, increased blood levels of Interleukin 1, decreased blood levels of cortisol (Abrams, 2001), heart rate , skin conductance, muscle activity; anxiety and depression, blood pressure, and subjective stress in human and rodent subjects (Nunez et al., 2002; le Roux, Bouic, & Bester, 2007; Suda et al., 2008; Chafin, Roy, Gerin, &

Christenfeld, 2004; Byrnes, 1996; Lehrer, Woolfolk, & Sime, 2007). The present research used a classical music selection for several purposes: (1) it would be difficult to determine a musical preference in animals; (2) self-selected music would be difficult to control experimentally; (3) classical music is commonly used and reported; and (4) if one genre of music is effective, then treatment options in medical settings could be less expensive and logistically easier to administer.

Mozart Effect

The present study used classical music selections from W.A. Mozart. Mozart pieces, specifically, were chosen based on the evidence in the research literature involving the use of music. The Mozart effect is a term generally used to refer to the use of music, specifically music composed by Mozart, to improve health. Initially, the Mozart effect referred to the use of music to improve learning. In 1993, Rauscher, Shaw, and Ky exposed 36 college students to either 10 minutes of listening to a relaxation tape, music of Mozart, or silence, and performed three sets of standard IQ spatial reasoning tasks. Spatial IQ scores were 8-9 points higher in the “Mozart condition” than in the other conditions (Rauscher et al., 1993). Pulse did not change, suggesting that arousal was not the cause of the spatial score increases and the enhancing effects lasted 10 - 15 minutes (Rauscher et al., 1993). Rauscher, Shaw, and Ky (1995) exposed 79 students to similar conditions and reported improved performance in a paper folding and cutting task in the Mozart condition. Rauscher et al. (1995) suggested that early music training provides long term enhancement of nonverbal cognitive abilities. They hypothesized that listening to

Mozart may organize cortical firing patterns, provide exercise for exciting and priming cortical firing patterns, and enhance and facilitate cortical symmetry operations among inherent patterns (Rauscher et al., 1995). Rauscher (1999) concluded that the Mozart effect increases spatial-temporal reasoning test scores, regardless of musical preference, better than relaxation instructions and is not caused by changes in emotion or arousal. Jones and Estell (2007) reported that listening to Mozart increased spatial reasoning with no differences in arousal in a study with 86 high school students. Hetland (2000) performed a meta-analysis of 36 studies with 2465 subjects combining spatial measures and 31 studies with 2089 subjects using spatial-temporal measures and found significant improvements when exposed to Mozart.

Some researchers have suggested that the Mozart effect may work through its impact on arousal. Thompson, Schelenberg, and Husain (2001) tested 24 undergraduate/graduate students, and the students exposed to Mozart increased spatial scores on paper folding and the cutting subtest of the Stanford Binet and had an increase in mood and arousal. However, when mood and arousal were controlled, there was no effect on spatial scores (Thompson et al., 2001). Jones, West, and Estell (2006) tested the Mozart effect of increased spatial ability in 41 college students using a spatial relations subtest. They reported a positive effect when listening to Mozart, where arousal mediated this association possibly by optimizing mood (Jones et al., 2006). Jausovec and Habe (2003) exposed 18 individuals to 3 minutes listening to compositions of Mozart, Brahms, or Haydn. Individuals exposed to Mozart had consistently lower

alpha band brain waves, and less pronounced brain wave gamma bands than the other group suggesting that Mozart influences levels of arousal (Jausovec & Habbe, 2003). Chabris (1999) performed a meta-analysis of 20 studies and found a small cognitive improvement with Mozart exposure. He suggested that this improvement may be the result of right cerebral hemisphere activation, responsible for spatial tasks and arousal.

Other research has suggested that the Mozart effect works by influencing brain activity. Jausovec, Jausovec, and Gerlic (2006) exposed 56 participants to 8 minutes of Mozart, and these participants performed better on a spatial rotation task, had less complex electroencephalographic (EEG) patterns, and had neural (cortical) activation than those not exposed to Mozart. In 2001, Jenkins studied 3 and 4 year olds for 6 months and reported that exposure to Mozart pieces improved spatial-temporal reasoning tests by 30%, enhanced synchrony of electrical firing pattern in right frontal and left temparoparietal areas, increased beta spectrum of EEG in right temporal, left temporal, and right frontal regions. Jenkins (2001) concluded that Mozart music activated wide distributions of brain areas, and PET/fMRI showed activation in the prefrontal, temporal, parietal regions, regions involved in spatial-temporal reasoning that overlap with music processing. Bodner, Muftuler, Nalcioglu, and Shaw (2001) performed fMRI studies comparing cortical blood flow activation while listening to Mozart, Beethoven, or 1930s piano music. They reported significant differences in temporal cortex, dorsolateral prefrontal cortex, occipital cortex, and cerebellum activation (Bodner et al., 2001).

The Mozart effect also has been reported to alter seizure activity. Lahiri and Duncan (2007) reported a case study of a 53-year-old male with a history of gelastic seizures (seizures characterized by uncontrollable bursts of energy that are typically unresponsive to therapy). For 3 months, this patient listened to Mozart 45 minutes a day and had no secondarily generalized tonic-clonic seizures, decreased manifestation of gelastic seizures, no altered perception, and decreased epileptiform activity on the EEG while unconscious, suggesting that the conscious awareness and appreciation of music is not needed for the effects to occur (Lahiri & Duncan, 2007). Hughes (2002) suggests characteristics that may account for decreased seizure and epileptiform activity of Mozart exposure includes the repetition of melodic line and the long lasting periodicities compared to music by Bach, Wagner, Beethoven, Chopin, Liszt, and Hayden.

A few reviews of the Mozart effect have been performed. Shaw (2001) found that: (1) college students exposed to 10 minutes of Mozart had improved short-term (10 - 15 minutes) spatial-temporal reasoning; students who performed below average on pretest had the largest enhancement (60%); (2) Alzheimer patients showed enhanced short-term spatial-temporal reasoning after exposure to Mozart; (3) exposure to Mozart reduced neuropathological spiking activity in epileptic patients even in a coma; (4) long-term exposure to Mozart produced enhanced maze learning by rats and enhanced performance lasted more than 4 hours; (5) fMRI showed activation in cortical regions (temporal cortex), dorsolateral prefrontal cortex, occipital cortex, and cerebellum when listening to

Mozart. These brain regions are all important for spatial-temporal reasoning. Hughes (2001) found that exposure to Mozart: (1) improved performance of spatial IQ by 8 - 9 points in 36 undergraduates, increased EEG consistency, increased correlations of neurophysiological activity on the temporal and left frontal areas; (2) decreased epileptiform activity and seizures over time. Hughes (2001) suggested that the reasons for these effects are that Mozart has more major and minor peaks, shorter periodic changes (about 30 seconds), high long-term periodicity scores, greater subharmonic and harmonics, and higher repeating notes. Mozart may directly impact cerebral cortex function (not secondary to stimulation or emotion as shown by patients in a coma). There is decreased alpha activity associated with improved spatial testing, and there are repetitions and periodic changes found in all aspects of brain function and bodily functions that resonate well with the periodic changes of Mozart. Hughes and Fino (2000) analyzed 81 pieces of Mozart, 67 pieces by J.C. Bach, 67 pieces by J.S. Bach, 39 pieces by Chopin, and 148 pieces from 55 other composers, and they found that long term periodicity (especially 10 - 60 sec, mean and median of 30 seconds) were significantly more frequent in Mozart pieces compared to works of other composers.

The Mozart effect also has been reported to alter other biological functions. Fukui and Toyoshima (2008) reported that Mozart enhances synaptic plasticity, alters secretions of steroids, and alters cranial nerves. Zhu et al. (2008) reported that Mozart affects voluntary and involuntary attention measured by event related potentials. Escher and Evequoz (1999) found in an experiment

with 23 healthy young individuals wearing 24 hour Holter EKGs that exposure to Mozart resulted in significant reduction of heart rate and also significant reduction of heart rate variability.

It is important to note that not all research has reported a significant Mozart effect or has been able to replicate results of Rauscher and Shaw's (1998) seminal study (Schellenberg & Hallam, 2005; Lints & Gadbois, 2003; McCutcheon, 2000; Bridgett & Cuevas, 2000; Steele, Brown, & Stoecker, 1999). Some research has only reported Mozart effects in certain populations. For example, Gilleta, Vrbancic, Elias, and Saucier (2003) reported an enhancement of mental rotation task after listening to Mozart in women only, and Twomey and Esgate (2002) reported enhancement in a spatiotemporal task in only nonmusicians. The research examining the effects of listening to Mozart is far from complete and more research is necessary.

Mozart Effect in Animals

Although most of the research examining the Mozart effect has been performed in human participants, there are studies that suggest that the Mozart effect occurs in animals as well. Rauscher, Robinson, and Jens (1998) exposed Long Evans rats *in utero* plus 60 days post-partum to compositions of Mozart, compositions of Philip Glass, white noise, or silence. They were tested for five days in maze testing, and by day 3, the Mozart group completed the maze more rapidly and continued to increase speed by day 5 (Rauscher et al., 1998). In 2006, Rauscher exposed Sprague-Dawley rats once to Mozart or white noise for 12 hours during their dark cycle after weaning. The Mozart condition performed

the maze faster and with fewer errors (Rauscher, 2006). Rauscher (2006) examined mRNA and proteins in hippocampi and spinal cords and found a 150% increase in brain-derived neurotrophic factor (BDNF) (involved in molecular mechanisms underlying cognitive function), a 140% increase in synapsin 1, a 176% increase in cAMP response element-binding (CREB) mRNAs, and upregulation of several genes involved with synaptic function/plasticity and intracellular signaling in rats exposed to Mozart. Aoun, Jones, Shaw, and Bodner (2005) exposed mice to Mozart for 10 hours and 5 hours and performed maze testing 6 hours or 24 hours after exposure. Mozart exposure significantly decreased work time and error in rats exposed to both 10 hours and 5 hours. Lemmer (2008) exposed rats to Mozart for 2 hours at 75 dB. Using radiotelemetry to measure cardiovascular function, exposure to Mozart significantly decreased heart rat in spontaneously hypertensive rats during the light cycle (Lemmer, 2008). It appears that exposure to Mozart has profound effects in many areas of functioning and, therefore, may be relevant to stress responses that may be associated with cancer.

Music and Cancer

Critically ill patients are often too sick to engage in conventional cognitive behavioral therapy with regular sessions and behavioral activation (Magill, Levin, & Spodek, 2008). Listening to music improves quality of life of cancer patients (Bozcuk et al., 2006). Oncology patients often use music to reduce stress, improve mood, improve fatigue, decrease fear, facilitate communication, inspire reflection, strengthen faith, improve insomnia, improve appetite loss, decrease

nausea and vomiting, reduce blood pressure, improve quality of life, decrease cortisol, and decrease pain (Kemper et al., 2008; Magill, Levin, & Spodek, 2008; Bozcuk et al., 2006; Hilliard, 2003; Joske, Rao, & Kristjanson, 2006; Burns, Harbuz, Hucklebridge, & Bunt, 2001; Richardson, Babiak-Vazquez, & Frenkel, 2008; Ferrer , 2007). In addition, listening to music significantly decreases anxiety, depressive symptoms, and physical discomfort in cancer patients (Joske, Rao, & Kristjanson, 2006; Cai, Li, & Jiao, 2001). Music generally decreases anxiety in stressful medical conditions, and improves side effects of chemotherapy and the well-being of adult and child oncology patients (Bozcuk et al., 2006). Music also reduces symptom severity for people with cancer, reduces discomfort, increases physical vigor and endurance, and increases physical relaxation via the endogenous production of pain-inhibiting beta endorphins as a part of pleasure or thrill responses (Abrams, 2001). Nunez et al. (2002) reported that listening to classical music reduced suppressive effects of stress on immune parameters in mice and decreased the enhancing effects of stress on the development of lung metastasis provoked by carcinosarcoma cells. Classical music enhanced the immune parameters and the antitumor response in unstressed rodents. These findings suggest that music can attenuate or possibly reverse adverse effects of stress on the number and function of lymphocytes that are required for an optimal response against cancer in rodents (Nunez et al., 2002). However, no study has examined effects of a classical music intervention and a noise control condition with stress in a breast cancer animal model.

Benefits of Animal Models

The use of animal models in research has substantial benefits. Animal models allow increased experimental control, logistically favorable time tables, and the ability to conduct experiments that would not be considered ethical in human research. For the present research project, manipulating a chronic stressor and giving subjects cancer is unethical in humans. Further, breast cancer progression takes decades in humans but can be studied over months in rats. Another advantage of using an animal model concerns the experimental manipulation of music exposure as the environment is completely controlled.

Utilization of an animal model in the present research also has benefits in terms of the dependent variables. In humans, requiring repeated measurements over weeks is taxing for the subjects, logistically difficult, expensive, and often results in missing data. In an animal study, the subjects are always available. Further, the data collection procedures can be carefully controlled, including time and environmental conditions (e.g., temperature, humidity, exposure to sounds, and other environmental exposures), which may influence the results (Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010).

1-methyl-1nitrosourea (MNU) Rat Model of Mammary Cancer

An animal model of chemically-induced mammary cancer in rats was used in the present research. 1-methyl-1-nitrosourea-induced mammary carcinomas (MNU) is one of the most widely used experimental animal models for breast carcinogenesis, and it has contributed to the understanding of the biology of

breast cancer and to potential preventive approaches (Lu, Jiang, Mitrenga, Cutter, & Thompson, 1998; Thompson, McGinley, Rothhammer, & Singh, 1995). It is used to evaluate preventive and therapeutic agents for human breast cancer and to study cancer progression (Perse, Cerar, Injac, & Strukelj, 2009). The MNU model of mammary cancer is a simple method that allows the rapid induction of premalignant and malignant stages of mammary carcinogenesis in the rat (Thompson et al., 1995). It is easier to implement and reproduce than other chemically-induced mammary cancers (Lu et al., 1998).

The primary distinctions between this model and other chemically-induced models in the rats is that: (1) MNU is injected in the rat at 21 days of age rather than 50 days of age; (2) an experiment can be completed within 35 days of carcinogen administration rather than being carried out for 6 months because mammary glands are less complex in younger animals; and (3) it is possible to detect and to quantify the occurrence of premalignant and malignant mammary gland lesions using this model (Thompson, Singh, & McGinley, 2000). Injecting rats at around 21 days induces a large tumor response over a short time interval with no evidence of toxicity (Thompson et al., 1995). In fact, the detection of mammary carcinomas in all MNU carcinogen treated rats is approximately 5 weeks and no later than 8 weeks (H.J. Thompson, personal communication, July 7, 2009; Thompson, McGinley, Rothhammer, & Singh, 1998; Thompson, Singh, & McGinley, 2000). Significant palpable tumor response is typically observed 30 days post carcinogen administration (Thompson et al., 1995). MNU injections

are reported to result in >99% incidence of palpable mammary gland tumors that are malignant (Thompson et al., 1995; Thompson, Singh, & McGinley, 2000).

The pattern of MNU-induced mammary cancer occurrence observed in the rat is consistent with the pathogenesis of the disease reported in humans (Thompson, Singh, & McGinley, 2000). Substantial evidence suggests that the MNU animal model is similar to human breast cancer in many aspects (Perse et al., 2009). Both human breast cancer and MNU mammary cancer in rats are predominantly ductal carcinomas (Thompson et al., 1995). The proportion of mammary carcinomas that are ovarian-hormone dependent in the MNU model is similar to that observed in the human disease, and the proportion of non-ovarian-hormone dependent mammary carcinomas is similar to that observed in the human disease as well (Lu et al., 1998; Christov, Grubbs, Shilkaitis, Juliana, & Lubet, 2007).

The carcinomas induced by MNU tend to be aggressive and locally invasive and have clear operational distinctions between the initiation and promotion stages of the disease process (Lu et al., 1998). The initiation stage is when there is a transformation of a normal mammary ductal cell into a tumor cell when the carcinogen is administered (Welsch, 1992), and the promotion stage is the time period after carcinogen administration when the tumorous mammary cells grow (Welsch, 1992). Therefore, the MNU model of mammary cancer in rats is appropriate to study risk factors during the promotion and progression stages of mammary carcinogenesis.

Relevant Variables to the Present Research

The present research was a 2 x 3 full factorial design. The independent variables included recurrent stress (or no stress) and music exposure (or noise exposure or no music/noise exposure). Subjects were 90 female rats that were injected with MNU to develop mammary carcinomas. The dependent variables were biological and behavioral variables relevant to breast cancer progression and stress responses. The biological dependent variables were: time until first tumor detection, tumor incidence, tumor multiplicity, tumor weight, body weight, adrenal gland weight, spleen weight, and serum corticosterone. The behavioral dependent variables were: food consumption, locomotor center time activity (an index of anxiety), locomotor horizontal activity and locomotor vertical activity (an index of depression), and ultrasonic vocalizations (an index of positive and negative affect).

Independent Variables

Stress. Immobilization stress is a painless stressor that had been effectively used in stress investigations with rats (Faraday, 2005; Shafer, 2006; Hamilton, 2010). This commonly used stress manipulation reliably elicits behavioral and biological stress responses in rodents, including elevations in hypothalamic-pituitary-adrenocortical (HPA) hormones, enlarged adrenal glands, and decreased thymus and spleen weights (Kant, Leu, Andersen, & Mougey, 1987; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992; Plotsky & Meaney, 1993; Acri, 1994; Faraday, O'Donoghue, & Grunberg, 1999; Faraday, 2002; Silverman, Pearce, Biron, & Miller, 2005; Herzog et al., 2009). It can be used as

a single acute stressor, or it can be repeated. In the present experiment, immobilization stress was combined with an unpredictable stressor and administered three times per week for eight weeks to mimic recurrent stressors that people may commonly experience. Unpredictable, painless stressors included noises, light flickering, and restraint shaking. Unpredictable stressors are a face-valid model of human stressors and have reliably produced alterations in stress hormones (Fride, Dan, Feldon, Halevy, & Weinstock, 1986; Weinstock, Matlina, Maor, Rosen, & McEwen, 1992) and behavior (Fride et al., 1986; Gonzalez Jatuff, Berastegui, Rodriguez, & Rodriguez Echandia, 1999) in rodent studies. In addition, various unpredictable stressors combined with immobilization restraint stress should decrease the rodents' habituation to the stressor and keep the stress manipulation novel.

Music. Music can be a noninvasive and cost effective form of stress management (Erken et al., 2008). Most of the literature on music as a stress reducer suggests that the use of self-selected and classical music can effectively reduce stress in humans (Erken et al., 2008; Clark et al., 2006; Akombo, 2007; Labbe et al., 2007). For the present research classical music was used based on the stress-reducing effects found in the literature (Erken et al., 2008; Clark et al., 2006; Nunez et al., 2002), and because determination of a rat's musical preference would be difficult. Music that has anxiolytic qualities tends to be repetitive in rhythm, has predictable dynamics, low pitch, slow tempo, and lack of percussion and vocals (Watkins, 1997). For the present research Mozart pieces were selected based on these initial criteria and because studies report biological

and behavioral effects from exposure to Mozart in rats (Rauscher, 2006; Rauscher, Robinson, & Jens, 1998; Lemmer, 2008; Aoun, Jones, Shaw, & Bodner, 2005) (See Methods section for details about music selection for the present research).

Dependent Variables

Tumor Measures. There are various tumor measures that can be examined to determine cancer progression. Four measures were included in the present experiment: time until first tumor detection, tumor incidence, tumor multiplicity, and end tumor weight (H.J. Thompson, personal communication, July 13, 2009). The occurrence of mammary tumors was detected by palpation of the mammary glands of each rat (Thompson, 2000). Time of first tumor detection was when the first mass was detected by palpation in each animal. Tumor incidence measured the number of animals in each experimental condition that had developed a tumor(s). Tumor multiplicity measured the number of tumors per animal detected by palpation throughout the experiment and was confirmed at the end of the experiment after rats were euthanized. End tumor weight measured the weight of all masses per animal removed after the rat was euthanized. All of these measures were used as gross indicators of tumor progression. (See Methods section for details about tumor measure procedures.)

Body weight and food consumption. Body weight and food consumption were measured as general indicators of animal health and growth (Faraday, 2005; Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010;

Hamilton, 2010). These variables were included in the present experiment because stress affects body weight and food consumption (Faraday, 2002; Shafer, 2006; Berger, 2009; Grunberg & Straub, 1992) and breast cancer can be affected by body weight. Specifically, excessive body weight is a known risk factor for breast cancer (ACS, 2007). Fat cells in the body produce small amounts of estrogen and some breast cancers are nourished by estrogen (ACS, 2007).

Corticosterone. Corticosterone (cortisol in humans) is released in response to a stressor (Faraday, 2005; Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010). Increases in corticosterone are commonly used as an indicator of stress responses in rats (e.g., Faraday, Blakeman, & Grunberg, 2005; Kalinichev et al., 2002; Kant et al., 1987; Hayley, Borowski, Merali, & Anisman, 2001; Faraday, 2005; Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010). Similar to cortisol in humans, a corticosterone increase/release in response to a stressor is healthy when the response is short-lived, but if corticosterone release is prolonged, it can be maladaptive and associated with physical and psychological health problems (e.g., McEwen, 1998).

Stress reliably changes levels of corticosterone. Restraint stress used on rodents elevates corticosterone (Acri, 1994; Kant, Leu, Andersen, & Mougey, 1987; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992; Faraday, 2005; Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010). In fact, increased serum corticosterone levels have been

reported after 14 days of 20 minute restraint (Faraday, Blakeman, & Grunberg, 2005). Corticosterone was used in the present experiment as an indicator of HPA activation in response to stress.

Tissue Measures. Tissues such as the spleen and adrenal glands are affected by stress and are involved in immune function. The adrenal glands are part of the hypothalamic-pituitary-adrenal axis. When responding to a stressor, the adrenal glands secrete catecholamines (norepinephrine and epinephrine) and corticosteroids (Guyton & Hall, 2000). Lymphocytes, which are critical to immune function, are located in the spleen and thymus (Guyton & Hall, 2000). The spleen manufactures lymphoid cells and produces macrophages that are an important line of defense in the immune system (Guyton & Hall, 2000). Stress has been reported to enlarge adrenal glands and decrease spleen weights (Silverman et al., 2005; Herzog et al., 2009). Spleen and adrenal gland weights were measured as gross indicators of the stress response and immune function.

Locomotor activity. A rat's movement when put in a non-home cage area is considered open field locomotion (Faraday, 2005; Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010). Locomotor activity can be an index of an animal's general health and activity and a measure of simple learning (e.g., habituation to a novel environment). Time spent exploring and investigating can be an index of depression, and time spent in the center of the chamber can be an index of anxiety (Faraday, 2000; Hlavacova & Jezova, 2008; Pohorecky, 2008; Sarkisova, Kulikov, Midzyanovskaya, & Folomkina, 2008; Zhuang, Xu, & Chun-Zhi, 2007; Grippo, Beltz, & Johnson, 2003; Faraday,

2005; Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010). Locomotor measures are commonly used in rodent experiments. Locomotor activity was included in the present experiment to index simple learning, depression, and anxiety. The center time is inversely related to anxiety because the rat's natural tendency is to remain along the outside of a novel environment rather than being in the center because the walls provide a level of protection from a potential external threat (Faraday, 2005; Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010). As the rat spends more time in the center of the open field, it is hypothesized to reflect less anxiety because it is comfortable being exposed in the center of the open field (Faraday, 2005; Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010). Horizontal activity and vertical activity are included to index depression-like behaviors. Horizontal activity and vertical activity measure exploratory and investigative activity. It is hypothesized that decreased exploration and investigative activity can be interpreted as depressive behavior (e.g., lack of interest) (Sarkisova, Kulikov, Midzyanovskaya, & Folomkina, 2008; Zhuang, Xu, & Chun-Zhi, 2007; Grippo, Beltz, & Johnson, 2003).

Stress has been reported to decrease open-field activity in rats (Faraday, 2002; Galea, Wide, & Barr, 2001). Increased center time has been interpreted as decreased anxiety and decreased center time is interpreted as increased anxiety (Beck & Luine, 2002; Gamallo, Villanua, Trancho, & Fraile, 1986; Lee, Tsai, & Chai, 1986; Faraday, 2005; Shafer, 2006; Berger, 2009; Perry, 2009;

Long, 2010; Starosciak, 2010; Hamilton, 2010). This project examined the effects of stress and music exposure on anxiety-like behavior as indexed by center time, depressive behavior as indexed by horizontal and vertical activity, and simple learning as indexed by within-session horizontal movement habituation.

Ultrasonic Vocalizations. Ultrasonic vocalizations provide an index of both positive and negative affect. Rats vocalize at different frequencies when responding to various stimuli (Long, 2010; Burgdorf & Panksepp, 2006; Panksepp, 2007; Panksepp & Burgdorf, 2000; Panksepp & Burgdorf, 2003). Stimuli considered positive, such as grooming by mothers and playing, generally cause rats to produce 50 kHz (frequencies between 35 – 96 kHz) vocalizations (Burgdorf & Panksepp, 2006; Panksepp, 2007; Panksepp & Burgdorf, 2000; Panksepp & Burgdorf, 2003; Long, 2010). Stimuli considered negative, such as receiving a foot shock or being placed in a novel situation, cause rats to produce 22 kHz (frequencies between 15 – 35 kHz) vocalizations (Brudzynski, Ociepa, & Bihari, 1991; Rosa, Nobre, Oliveria, & Brandão, 2005). Based on Long (2010), Burgdorf and Panksepp (2006), and Brudzynski, Ociepa, and Bihari (1991), 50 kHz ultrasonic vocalizations were considered to be a measure of positive affect, whereas 22 kHz ultrasonic vocalizations were considered to be a measure of negative affect.

PRELIMINARY PREPARATIONS

A preliminary study was conducted to evaluate a method of manipulating recurrent stress. A common and effective method of manipulating stress is to

use restraint stress (Kant, 1987; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992, Acri, 1994; Faraday 2002), a finger-like restraining device that holds the animal still. Previous experiments have used this method of stress during a two to three week stress period, whereas the present experiment had an eight week stress period. In restraint stress, the "fingers," are tightened until the subjects are completely immobile but not in pain (Shafer, 2006; Faraday, 2005; Hamilton, 2010). Another immobilization technique is to use a broome-style rodent restrainer, a clear, acrylic cylinder that comes in various sizes depending on the size of the rodent. The rodent is immobilized by adjusting a plastic cone to shorten the amount of space available in the cylinder until the rodent is immobile; this is effective at inducing stress hormones (Laugero, Gomez, Manalo, & Dallman, 2002; Chang & Opp, 2002). Both types of restrainers were used in this preliminary experiment to prevent habituation and to optimize the novelty of the restrainers. In addition, the broome-style restraints were used initially because the rodents were too small (less than 50g) for the finger-like restraint (e.g., rats would be able to escape). This preliminary experiment was conducted to insure that the exposure to restraint stress and other unpredictable stressors produced a stress response during an eight week stress period. Six female Sprague-Dawley adolescent rats (e.g., beginning at 21 days old) were exposed to restraint stress and other unpredictable stressors (noise, light, cage shaking) for 20 minutes a day, three times a week, for six weeks based on a similar study by Hamilton (2010). A control group of six rats was exposed to 2 - 3 minutes of handling to ensure that any corticosterone effects were the result of the restraint

stress and unpredictable stressors and not handling. Using the data from this preliminary study, the present study's sample size was adjusted to have sufficient power to detect significant differences in stress responses.

Prior to the present experiment, the investigator visited the laboratory of Henry Thompson, Ph.D., at Colorado State University. Dr. Thompson developed and is expert in the MNU-induced mammary cancer rat model. During this visit, the investigator worked with Dr. Thompson and learned how to prepare MNU, administer MNU via I.P. injections, palpate rats in the laboratory that already had tumors from the MNU paradigm, excise tumors during necropsy, prepare tumors for histopathology, and deactivate MNU. (See the Methods section for details of these procedures.)

In addition, before starting the present experiment the investigator consulted with Environmental Health and Safety (EHS) at USUHS. After receiving the MNU Material Safety Data Sheet, the investigator performed a respirator fit-test before handling the chemical. Also, EHS performed a safety inspection on the chemical fume hood to make sure the hood's airflow was satisfactory.

Because this doctoral research had elements (e.g., mammary cancer) outside the realm of her major advisor's research areas and ongoing, approved research protocols, a full protocol was written and submitted to IACUC for review and approval. The present experiment's protocol was approved in January 2010. A copy of protocol's IACUC approval memorandum appears in Appendix A.

Dr. Frances Rauscher from the University of Wisconsin Oshkosh, was consulted regarding the Mozart musical pieces. Dr. Rauscher is an expert in research regarding the *Mozart Effect* and authored the seminal study that began research in this topic area. In addition, Dr. Rauscher has tested the *Mozart Effect* in rat as well as human subjects (for more details see the Methods section).

OVERVIEW OF EXPERIMENT

The present experiment evaluated effects of recurrent stress and music exposure on behavioral and biological variables often associated with the early natural history of breast cancer. Collection of data was approximately ten weeks. This research was the first to evaluate the effects of stress on time until first tumor detection, tumor progression including size, incidence, and multiplicity, and secondary stress measures associated with exposure to stress and the development of tumor pathology using the MNU-induced mammary cancer rat model (H.J. Thompson, personal communication, July 7, 2009). This research also provided detailed description of the type of music exposure utilized (which is often not provided in previous music research) to provide a clearer direction for future research examining the effect of music exposure. Past research involving music exposure as a form of stress reduction has been vague by either providing no description of the music utilized or by only including the name of the musical piece or genre. The present research included genre, names of the musical pieces, and an analysis of the music's characteristics. In addition, the present research included a noise condition to serve as a sound control that is rarely

included in past research. The experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee (IACUC) and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (National Institutes of Health [NIH], 1996).

HYPOTHESES

The present experiment used rats to examine effects of recurrent stress on subsequent behavioral and biological variables relevant to breast cancer progression. The experiment also examined if a stress management intervention (music exposure) attenuates any detrimental effects of stress. The experiment was a 2 (stress or no stress) x 3 (music exposure, noise exposure, or no music/noise exposure) full factorial design. The goals of the experiment were to determine: (1) behavioral and biological effects of stress on tumor progression; (2) whether music exposure attenuates effects of stress; and (3) whether music exposure attenuates effects of stress on tumor progression.

There were six major hypotheses based on the domains of the dependent variables: (1) body weight/food consumption; (2) measures of stress and immune function (i.e., corticosterone, spleen weight, adrenal gland weight); (3) tumor measures (time until first tumor detection, incidence, multiplicity, and end tumor weight); (4) locomotor activity (index of simple learning); (5) index of anxiety (locomotor center time); and (6) index of depression (locomotor horizontal and vertical activity) and negative affect (ultrasonic vocalizations).

Hypothesis 1: Body Weight and Food Consumption

Hypothesis 1a

Stress will increase body weight and food consumption. Body weight and food consumption can increase in response to stress, particularly in females when exposed to stressors including noise stressors (which are aspects of the unpredictable stressors that were used)(Greeno and Wing, 1994; Grunberg and Straub, 1992; Kawanishi, Fukuda et al., 1997; Krebs et al., 1996).

Hypothesis 1b

Exposure to music will attenuate the body weight gain and food consumption. There is currently no literature on the effects of music exposure on body weight and food consumption.

Hypothesis 1c

Exposure to music will attenuate the effects of stress on body weight and food consumption compared to the noise exposure and no music/noise control group. While there is currently no literature on the effects of music on body weight and food consumption, this hypothesis was based on the evidence that music can attenuate some effects of stress (Erken et al., 2008; Nunez et al., 2002; Avers et al., 2007).

Hypothesis 2: Measures of Stress

Hypothesis 2a

Stress will increase serum corticosterone in rats. Previous research reports that stress manipulations, including restraint stress and other unpredictable stressors, increase serum corticosterone (e.g., Kant et al., 1987;

Raygada et al., 1992; Plotsky & Meaney, 1993; Acri, 1994; Faraday, et a., 1999; Faraday, 2002, Fride et al., 1986; Weinstock et al., 1992; Shafer, 2006; Berger, 2009; Long, 2010; Perry, 2009; Starosciak, 2010; Hamilton, 2010). Stress will increase adrenal gland weights and decrease spleen weights. Previous research reports that stress manipulations enlarge adrenal glands and decrease spleen weights (Silverman et al., 2005; Herzog et al., 2009).

Hypothesis 2b

Exposure to music will lower corticosterone levels, attenuate adrenal gland weight, and increase spleen weights compared to exposure to noise or no music/noise. Previous research reports that music exposure can decrease biochemical measures of stress (Nunez et al., 2002; Watkins, 1997; Joske et al., 2006; Smolen et al., 2002; Rao et al., 2007; Abrams, 2001).

Hypothesis 2c

Exposure to music will attenuate stress effects on corticosterone levels and spleen and adrenal gland weights. This hypothesis was based on evidence that stress increases corticosterone levels and music exposure can decrease corticosterone levels (Kant et al., 1987; Raygada et al., 1992; Plotsky & Meaney, 1993; Acri, 1994; Faraday, et a., 1999; Faraday, 2002, Fride et al., 1986; Weinstock et al., 1992; Nunez et al., 2002; Watkins, 1997; Joske et al., 2006; Smolen et al., 2002; Rao et al., 2007; Abrams, 2001).

Hypothesis 3: Tumor Measures

Hypothesis 3a

Stress will decrease the duration until detection of first tumor occurrence, increase the number of animals that develop tumors (incidence), increase the number of tumors present (multiplicity), hasten tumor growth, and will have larger tumors/tumor spread (end tumor weight) than animals not exposed to stress. Previous research reports that stress is associated with promoting tumor growth, tumor burden, and metastasis (Azar, 1999; Sklar & Anisman, 1980; Giraldi et al., 2000; Bruzzone et al., 2008; Ross, 2008; Steplewaki et al., 1985). To date, no research has examined the effects of chronic stress on tumor progression in the MNU-induced model of mammary cancer in rats. Because all rats were injected in the proposed experiment, it was hypothesized that tumor occurrence would appear sooner and would progress at a faster rate with stress than with no stress.

Hypothesis 3b

Exposure to music will attenuate time until incidence of first tumor, will decrease tumor incidence, will decrease tumor weight, will attenuate tumor growth, and will decrease tumor numbers. No studies have examined effects of classical music on these measures in a rat model of breast cancer. This hypothesis was based on the decreased number of tumor nodules found in a rat model of lung cancer when exposed to music (Nunez et al., 2002).

Hypothesis 3c

Exposure to music will attenuate the effects of stress on time until incidence of first tumor, will decrease tumor incidence, will decrease tumor weight, will attenuate tumor growth, and will decrease tumor numbers. No studies have examined effects of classical music on these measures in a rat model of breast cancer. This hypothesis was based on the decreased number of tumor nodules found in a rat model of lung cancer when exposed to music and stress (Nunez et al., 2002).

Hypothesis 4: Open Field Locomotor Behavior

Hypothesis 4a

Stress will alter activity levels in the locomotor open field chamber compared with rats not exposed to stress. Previous research reports that stress decreases horizontal activity open field chamber (Faraday, 2002; Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Hamilton, 2010; Starosciak, 2010). In addition, stress may interfere with within-session activity habituation (measure of simple learning).

Hypothesis 4b

Exposure to music will increase locomotor activity compared with exposure to noise or no music/noise and enhance within-session activity habituation. This hypothesis was based on the idea that if classical music is a stress management technique, it would affect locomotor activity like another stress reduction technique.

Hypothesis 4c

Exposure to music will attenuate the effects of stress on locomotor activity and within-session activity habituation. This hypothesis was based on literature that suggests that music can alter some effects of stress (Erken et al., 2008; Nunez et al., 2002; Avers et al., 2007).

Hypothesis 5: Anxiety-Like Behavior

Hypothesis 5a

Stress will increase anxiety-like behaviors (as assessed by decreased center time in an open field chamber) compared with rats not exposed to stress. Previous research reports that stress decreases center time in open field (Adamec, Head, Blundell, Burton, & Berton, 2006; Benaroya-Milshtein et al., 2004; Imanaka, Morinobu, Toki, & Yamawaki, 2006; Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Hamilton, 2010; Starosciak, 2010).

Hypothesis 5b

Exposure to music will decrease anxiety-like behaviors (increase center time). Previous research reports suggest that music decreases anxiety (Erken et al., 2008; Nunez et al., 2002; Avers et al., 2007).

Hypothesis 5c

Exposure to music will attenuate the effects of stress on anxiety. Human research has reported that social support expedites treatment for anxiety disorders (Lehrer et al., 2007; Watkins, 1997; West, 2004) and exposure to music is another form of stress management.

Hypothesis 6: Depression-Like Behavior

Hypothesis 6a

Stress will increase depressive-like behaviors (as assessed by negative affect determined by ultrasonic vocalizations and decreased horizontal and vertical activity in the locomotor chamber) compared with rats not exposed to stress. Previous research has reported that aversive stimuli increases negative affect in rats and decreases horizontal and vertical activity in the locomotor chamber (Brudzynski et al., 1991; Rosa et al., 2005; Grippo, Beltz, & Johnson, 2003; Long, 2010).

Hypothesis 6b

Exposure to music will decrease depressive-like behaviors compared with exposure to noise or no music/noise. Human research has reported that music can decrease negative emotions (Labbe et al., 2007; Akombo, 2007; Suda et al., 2008; Munro & Mount, 1978; Aitini et al., 2007; Long, 2010).

Hypothesis 6c

Exposure to music will attenuate the effects of stress on depressive behaviors. To date, no research has examined the effects of music on the effects of stress on depression. This hypothesis was based on the human reports that music exposure decreases both stress and negative emotions while promoting positive mood (Labbe et al., 2007; Akombo, 2007; Suda et al., 2008; Munro & Mount, 1978; Aitini et al., 2007; Long, 2010).

METHODS

The purpose of the present research was to examine the biological and behavioral effects of recurrent stress and music exposure on variables relevant to breast cancer. The subjects were 90 Sprague-Dawley female rats. The experiment was a 2 x 3 full factorial repeated-measures design with stress (no stress) and music exposure (or noise exposure or no music/noise exposure) as the independent variables. Rats were randomly assigned to a stress or no stress condition and to music, noise, or no music/noise exposure condition. Rats arrived at 20 days old, at which they were assigned three to a cage, as per the recommendation of the MNU-induced mammary cancer protocol to optimize MNU-induced carcinomas (H.J. Thompson, personal communication, July 13, 2009). The rats were handled on the day of arrival to help acclimate the rats to human touch and to reduce potential stress reactions from handling. On the second day, all the rats were administered MNU (see MNU Preparation and Administration for detailed procedure) and were not excessively handled for seven days (gentled for two more days and handled for weighing and tail marking). It was important that the rats did not start behavioral measures for seven days after MNU administration because this period of time, also called the initiation phase of carcinogen, was necessary for DNA repair and recovery (H.J. Thompson, personal communication, July 13, 2009). Over the next nine weeks (time necessary for tumor occurrence), the rats were acclimated to measures and assessed for biological and behavioral dependent variables relevant to breast cancer.

Determination of Sample Size and Power

The sample size (cell size of n = 15) was determined in two ways: (1) based on previous reports using similar dependent measures and responses to stressors (e.g., Shafer, 2006; Cohen et al., 2007; Pohl, Olmstead, Wynne-Edwards, Harkness, & Menard, 2007; Morrow, Redmond, Roth, & Elsworth, 2000; Perry, 2009; Berger, 2009; Long, 2010; Hamilton, 2010; Starosciak, 2010), and (2) a power analysis based on data from a preliminary study and a similar study (e.g., examined stress and music exposure on an animal model of lung cancer) by Nunez et al. (2002).

Studies in the research literature report statistically significant effects for various stressors (crowding, restraint, predator scent) with cell sizes of between 6 – 16 animals (e.g., Brown and Grunberg, 1995; Cohen et al., 2007; Day, Masini, & Campeau, 2004; Faraday, Elliott, Phillips, & Grunberg, 2003; Funk & Amir, 2000; Morrow et al., 2000; Perry, 2009). Measures of locomotor activity, body weight, food consumption, ultrasonic vocalizations, and corticosterone are well established in our laboratory and have shown significant effects and power of at least 0.80 in sample sizes as low as 8 subjects per cell (Perry, 2009; Berger, 2009; Long, 2010).

Because the present study used a combined stress manipulation, data from the preliminary study were used to determine sample size and power for serum corticosterone concentrations. Sample size and power were determined with a computer program, Java Applets for Power and Sample Size (Lenth, 2006-2009). The preliminary study found an effect for stress of 0.77 with an observed

power of 21% in a sample size of 6 rodents per group in a one-way ANOVA. Using the effect size of 0.77, power for the main effect of stress was calculated for a two-way ANOVA with a 0.05, two-sided significance. A cell size of 10 rats per group would allow for the detection of a main effect of stress to be observed at 83% power.

Because the present study was the first to examine the effects of stress and music exposure in an MNU-induced mammary cancer model, Nunez et al.'s (2002) was the most similar study (e.g., examined the effects of stress and music in a rodent model of lung cancer and found that stress exacerbated tumor nodules and music decreased stress response and number of tumor nodules), and therefore was used to help determine sample size and power. Nunez et al. (2002) found effect sizes for the interaction of stress and music ranging from 1.1 (for ACTH) to 8 (for number of tumor nodules) in a sample size of 10 rodents per group. Calculations using data from the Nunez group indicated that the observed effect size (Cohen's d) of the interaction between stress and music was 1.1 for ACTH. Using the effect size of 1.1, power for main effects and interactions were calculated. A cell size of 5 rats would allow for the detection of a main effect for stress with an effect size of 1.1 to be observed at 80% power with a significance level of $p < 0.05$. A cell size of 8 rats would allow for the detection of a main effect for music with an effect size of 1.1 to be observed at 80% power with a significance level of $p < 0.05$. A cell size of 23 rats would be needed to detect an interaction between stress and music at an effect size of 1.1 to be observed at 80% power with a significance level of $p < 0.05$. Because 23 rats per cell was

not feasible for the proposed research, sample size calculations were based on that which were needed to detect main effects of stress and music. However, in order for a significant interaction between stress and music to be observed at 80% power, with 15 rats per cell, the effect size would have to reach 1.45, which is possible based on the fact that the measure (ACTH) used by the Nunez group were comparable but are not the same as the measure (cort) that was used in the present research.

Calculations using data from the Nunez group indicated that the observed effect size (Cohen's d) of the interaction between stress and music was 8.0 for number of tumor nodules. Using the effect size of 8, power for main effects and interactions were calculated. A cell size of 15 rats would allow for the detection of a main effect for stress with an effect size of 8 to be observed at 100% power with a significance level of $p < 0.05$. A cell size of 15 rats would allow for the detection of a main effect for music with an effect size of 8 to be observed at 100% power with a significance level of $p < 0.05$. A cell size of 15 rats would be needed to detect an interaction between stress and music at an effect size of 8 to be observed at 100% power with a significance level of $p < 0.05$. Because the Nunez group found effect sizes of the interaction between stress and music ranging from 1.1 to 8 depending on the measure, we believe that 15 rats per cell would be sufficient. Calculations were based on a two-way ANOVA with a 0.05, two-sided significance level.

Based on the range of effect sizes in the literature, 8 animals per cell should detect significant main effects at 80% power with a significance level of

$p < 0.05$. However, the present research used 15 animals per group in order to be conservative, in case of death or illness of animals due to the nature of the study, and to maintain three animals per cage as suggested by the MNU-induced mammary cancer protocol.

Subjects and Housing

The subjects were 90 female Sprague-Dawley rats that were approximately 20 days old (Charles River Laboratories) at the beginning of the experiment. Sprague-Dawley albino rats were used because they are the most commonly used laboratory rats in stress studies and other experiments (Suckow, Weisbroth, & Franklin, 2006). In addition, female Sprague-Dawley rats at approximately 21 days old are the rats recommended for the MNU-induced mammary cancer model (Thompson, 2000). The experimental design included six conditions with 15 subjects per condition for a total of 90 rats. When they arrived, the rats were housed three per cage in polycarbonate cages (46 cm x 36 cm x 20 cm) on hardwood chip bedding (Pine-Dri) with continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water with no toys (to be comparable to other studies using the MNU model of mammary cancer). The housing room (for the subjects in the experiment) was maintained at room temperature of 23^0 C with a humidity of approximately 50% and a 12-hour (lights off 0100-1300) reverse light cycle with behavioral procedures taking place during the rats' active (dark) period. Housing conditions were designed to provide optimal levels of comfort to the rats within their home cages.

Upon arrival, subjects were randomly assigned to one of six conditions: (1) no stress + music exposure; (2) no stress + noise exposure; (3) no stress + no music/noise exposure (silence); (4) stress + music exposure; (5) stress + noise exposure; or (6) stress + no music/noise (silence) exposure. All subjects resided in the same housing room. The polycarbonate cages were changed and cleaned two times a week.

Independent Variables

Stress

There are many types of stressors used in animal experiments (e.g., electric shock, crowding, cold water immersion, predator, intruder, restraint, sleep disturbance). The present experiment used a variable stress paradigm that included exposure of rats to restraint stress, unpredictable noise (e.g., coins shaking, clapping, metal clanging), bright light flickering, and restraint device shaking in a setting that was separate from the housing room based on similar procedures used in our laboratory (Berger, 2009; Perry, 2009; Long, 2010; Hamilton, 2010; Starosciak, 2010). Restraint stress reliably produces a biochemical stress response in rats (Kant, 1987; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992, Acri, 1994; Faraday 2002; Shafer, 2006; Hamilton, 2010). The stress procedure occurred in a room that was not the housing room; the animals were transferred from their home cage to a restraint device for exposure to the stressors. The stress procedure lasted 20 minutes and occurred at various times during the rat's active cycle to prevent habituation to the stressors. A standard florescent overhead light remained on during this time. On

stress day 1, only the restraint device was used to induce stress. On the following stress days, additional stressors (e.g., bright light flickering, noises, or restraint device shaking) were presented along with use of the restraint device (see Table A for specific schedule of stressors). Additional stressors were presented three times within the 20 minutes of restraint, at the 5, 10, and 15 minute marks. The additional stressors (clapping, metal clanging, coin shaking, lights flickering, and restraint shake) were selected by day by using a research randomizing program (e.g., www.randomizer.org). The non-stress group remained in their housing room (in their home cages) while the stress group underwent the stress procedure. When the stress procedure ended, the experimenters changed lab coats and gloves (to minimize scent of stress hormones) and gentled the non-stress group. Both groups were in the housing room after the procedures. This stress procedure was based on a preliminary experiment that was conducted. The preliminary experiment included alternating restrainers (finger-trap and broome-like) with additional unpredictable stressors that were previously selected using a randomizer program. The female Sprague-Dawley rats were exposed to stressors three times a week for 8 weeks.

Table A. Stressor by Stress Day

Experiment Day	Type of Restraint	Additional Stressor
1	Broome-style restraint	White light only
2	Broome-style restraint	Clapping
3	Broome-style restraint	Coin shake
4	Broome-style restraint	Restraint shake

5	Broome-style restraint	Coin shake
6	Broome-style restraint	Metal clanging
7	Finger-trap restraint	White light flickering
8	Finger-trap restraint	Coin shake
9	Finger-trap restraint	Metal clanging
10	Finger-trap restraint	Restraint shake
11	Finger-trap restraint	Restraint shake
12	Finger-trap restraint	Clapping
13	Broome-style restraint	Clapping
14	Finger-trap restraint	Restraint shake
15	Broome-style restraint	Coin shake
16	Finger-trap restraint	Restraint shake
17	Broome-style restraint	Clapping
18	Finger-trap restraint	Metal clanging
19	Finger-trap restraint	White light flickering
20	Finger-trap restraint	Coin shake
21	Finger-trap restraint	Clapping
22	Finger-trap restraint	White light flickering
23	Finger-trap restraint	Metal clanging
24	Finger-trap restraint	Coin Shake

Music, Noise, or No Music/Noise Exposure

Music. The present experiment used musical pieces composed by W.A. Mozart that were burned onto a compact disc (CD) and played for a total of 5 hours (songs were played in a loop and stopped at 5 hours) to the music condition. The music exposure was based on previous animal studies (Aoun et al., 2005; Lemmer, 2008; Nunez et al., 2002). The musical pieces were based on Mozart selections used in previous animal research (personal communication with Dr. Frances Rauscher, November 22, 2009) as well as literature on Mozart selections and rat audiograms (Lemmer, 2008; Rauscher, 2006; Aoun et al., 2005; Rauscher et al., 1998; Hughes & Fino, 2000; Kelly & Masterton, 1977; Borg, 1982). Most research examining the Mozart effect has used the *Sonata for Two Pianos* (Aoun et al., 2005; Rauscher et al., 1998; Rauscher, 2006). Therefore, this selection was used as one of the selections of our musical recording because previous research has shown significant results using this piece. However, there has been controversy concerning this piece when used in rats because of the range of the rat audiogram (Steele, 2003).

Humans have a typical audiogram around the range of 20 Hz to 20 kHZ, although there is individual variation (Cutnell & Johnson, 1998). Rats, however, have a different range of hearing than humans. Kelly and Masterton (1977) determined the audiogram of Sprague-Dawley rats to be in the range of 250 Hz to 80 kHz at 70 dB using a conditioned suppression technique. Borg (1982), using a similar conditioned suppression technique, found audiogram results for

Wistar and Sprague-Dawley rats (albino) similar to those found by Kelly and Masterton (1977). In addition, Borg (1982) reported no sex difference between rats at a young age. Heffner, Heffner, Contos, and Ott (1994) performed a study to replicate Kelly and Masterton's (1977) results using pigmented, hooded Norway rats (Long Evans). They found that Long Evans rats had an audiogram range from 250 Hz to 70 kHz, suggesting that auditory sensitivity is not affected by albinism (Heffner et al., 1994). In addition, these audiograms were found in Long Evans rats that were 3 months old and 9 months old (Heffner et al., 1994).

Based on the literature on rat audiograms, in order to be conservative, the other musical selections chosen for music condition were of higher frequencies, such that more notes could be heard by the rat subjects. Based on the literature suggesting that musical selections (not just the *Sonata for Two Pianos*) by Mozart are unique compared to other classical composers in terms of periodicity scores and repetitions (Hughes & Fino, 2000; Hughes, 2001), have reported significant effects on heart rate (Lemmer, 2008), and after consultation with Dr. Frances Rauscher (personal communication, November 22, 2009), the use of several different Mozart selections for the present research was determined to be appropriate. Dr. Frances Rauscher indicated that different Mozart selections should be effective and that the *Sonata for Two Pianos* was used in multiple studies because it was the musical piece that was chosen for her first study (e.g., for the purpose of replication). The *Sonata for Two Pianos* (K. 448) contains approximately 60% of notes that are within the auditory threshold of rats (Rauscher, 2006). Using similar techniques as Rauscher (2006) five additional

selections by Mozart were selected for the present research. To ensure a greater number of notes were heard by rat subjects, the five additional selections met the criteria of containing no less than 70% of notes above 250 Hz.

All musical pieces were analyzed by David Garcia, a research collaborator and USUHS medical student who has music analysis experience to compare the musical selections with previous studies, to allow replication, and to provide parameters for the noise condition. The musical pieces used in the present experiment were: *Andante* in F for a *Small Mechanical Organ*, K. 616 (performed by Owen Murray), and was 6 minutes and 52 seconds long; *Concerto* in D for *Violin*, K. 211 (performed by Augustin Dumay and Camerata Academica Salzburg), and was 6 minutes and 58 seconds long; *Andante* in C for *Flute*, K. 315 (performed by Orpheus Chamber Orchestra and Susan Palma), and was 6 minutes and 15 seconds long; *Concerto for Flute and Harp* in C, K. 299 (performed by Berliner Philharmoniker, Fritz Helmis, Herbert von Karajan, and James Galway), and was 8 minutes and 18 seconds long; *Concerto* in G for *Violin "Stra[ss]burg"*, K. 216 (performed by Frank Peter Zimmermann, Jorg Faerber, and Wurttembergisches Kammerochester Heilbronn), and was 8 minutes and 59 seconds long; and *Sonata for Two Pianos Allegro*, K. 448 (performed by Christian Zacharias and Marie-Luise Hinrichs), and was 8 minutes and 2 seconds long. All pieces were played in a major scale.

For each piece of music used in the present study, music in the MIDI format was obtained. The MIDI format was then compared with a professional recording to determine the accuracy of the MIDI rendition. Music was then

analyzed using MIDINOTE (as was done in Rauscher [2006]). The MIDINOTE program took the music in MIDI format and created an output in a text file that was loaded into an Excel spreadsheet. The Excel spreadsheet added each notes' (A-G) temporal lengths for the 88 keys on a piano, and it added the total amount of note analyzed. Each note corresponds to a particular frequency. The total time for frequencies above 250 Hz was determined by adding all note times above and including middle C, which corresponds roughly to a frequency of 261 Hz. The middle C and above time was divided by the total analyzed time of the piece to yield a percentage of notes above (and including) middle C. The percentage of notes for each piece determined the selection of music for the present study. The analyses yielded the following percentages of notes above 250 Hz: *Andante* in F *for a Small Mechanical Organ*, 97%; *Concerto* in D *for Violin*, 77%; *Andante* in C *for Flute*, 75%; *Concerto for Flute and Harp* in C, 73%; *Concerto* in G *for Violin "Stra[ss]burg"*, 73%; and *Sonata for Two Pianos Allegro*, 61%.

The music selections were played for 5 hours after subjects were exposed to stress/no stress procedure via a portable CD player. Using a Larson Davis Model 2800 Sound Level Measurement Device, the sound was calibrated to play at approximately 65 - 70 decibels. After the stress + music condition was exposed to stress, the rats were returned to their home cages, placed into a different experimental room ("music" room) with the lights off, and exposed to the music. For the no stress + music condition, rats were taken into the "music"

experimental room in their home cages with the lights off and exposed to the music without prior exposure to stress.

Noise. The noise condition was necessary to provide a vital sound control that previous studies lack. The noise condition was created by taking broadband noise (e.g., pure white noise) and varying the intensities to similar parameters that were used in the music condition. The white noise also was calibrated and played at approximately 65 - 70 decibels. This condition served as a sound control intervention group and helped determine if any effects of music were the result of unique musical qualities or sound itself, perhaps because it may be a form of distraction. The white noise was recorded onto a CD and was played for 5 hours after a stress exposure for the stress + noise condition. For the stress + noise condition, the rats were returned to their home cages after stress exposure, placed into a different experimental room ("noise" room), and exposed to the noise. For the no stress + noise condition, the rats were taken into the "noise" experimental room in their home cages with the lights off and exposed to the noise without prior exposure to stress.

No Music/Noise (Silence). In this condition, the rats were taken into a different experimental room ("silent" room) in their home cages (with either prior stress exposure or no stress exposure depending on condition) with the lights off for 5 hours with no music or noise exposure. This condition served as a silence control group, to determine if any effects of the music or noise conditions were unique musical or noise qualities or exposure to an auditory stimulus.

Biological Dependent Variables

Body Weight

Body weight is relevant to many physical and mental health conditions (e.g., anxiety, depression, eating disorders, obesity) and is used in many rodent experiments as a measure of general health or to determine the effect of various manipulations on the animal (Suckow et al., 2006; Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Hamilton, 2010; Starosciak, 2010).

Body weight was measured two times a week for the entire experiment. Rats were taken from their home cages and placed in a weighing bowl on an electronic scale (Sartorious electronic scales). The electronic scale automatically took several readings during a 10 second time frame for each animal, and produced an average weight to take into account that the animals were not completely still and that movement could affect the weight (Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Hamilton, 2010; Starosciak, 2010).

Blood and Tissue Sample Collection

After the last experimental day, rats were sacrificed by Grunberg laboratory members by carbon dioxide inhalation followed by prompt decapitation via a rodent guillotine in accordance with current LAM procedures. Subjects were individually placed in a standard rat cage where they were administered 100% carbon dioxide (Airgas Puritan Medical, Exp. 01-24-2012) at a maximum rate of 10-20% of chamber volume per minute. The carbon dioxide was released between 3.0-4.0 L per minute into the rat cage via a special lid that was

connected to the carbon dioxide tank. Blood collection was based on previous procedures in the Grunberg Laboratory (e.g., Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Hamilton, 2010; Starosciak, 2010). Trunk blood was taken from the animals and immediately placed in non-heparinized tubes stored in a bucket of wet ice. Within 30 minutes of decapitation, the blood was spun in a refrigerated centrifuge (4º C) at 2500 rpm for 20 minutes. Serum was removed from the tubes via disposable pipettes and was placed into Eppendorf tubes and stored in a freezer at -80º C until assaying.

Immediately after decapitation, the rats' tumors were counted, recorded, and then removed from the body cavity using a scalpel and other medical instruments. The tumors were weighed on an analytical scale, measured with digital calipers, sliced, and then placed into a vial containing 10% buffered formalin phosphate. After 24 hours of being fixed in the formalin, tumor slices were rinsed and placed in vials containing 70% ethanol. Tumor slices were sent to Histoserv (Germantown, MD) for processing, embedding, and preparing hematoxylin and eosin (H & E) slides that were read by a veterinary pathologist to determine if tumors were malignant.

Immediately after decapitation, the rats' spleens and adrenal glands were removed from the body cavity by using a scalpel and other medical instruments. The tissues were weighed using an analytical balance and measurements were recorded.

Serum Corticosterone

A common biochemical marker of HPA axis activity in response to stress is corticosterone (Belz, Kennell, Czambel, Rubin, & Rhodes, 2003; Hennessy, 1997; Pham, Ickes, Albeck, Soderstrom, Granholm, & Mohammed, 1999; Selye, 1973; Brown & Grunberg, 1995; Faraday, 2002; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010). The corticosterone assay used for the present research was an ImmuChem Double-Antibody radioimmunoassay (RIA) kit purchased from MP Biomedicals. This procedure was performed in the laboratory of Neil E. Grunberg at the Uniformed Services University of the Health Sciences (USUHS) and procedures were based on previous work done in the laboratory (Berger, 2009; Perry, 2009; Long, 2010; Hamilton, 2010, Starosciak, 2010). According to Berger (2009), a limited supply of a specific antibody reacted with radioactive-labeled corticosterone competes with the free corticosterone from the samples. The amount of corticosterone was determined by measuring the radioactivity of the sample and comparing it to amount of radioactivity in known standards in a gamma counter (Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010).

Tumor Measures

Five different measures involving the tumors were taken in the present experiment to gauge tumor progression: time until first tumor detection, tumor incidence, tumor multiplicity, tumor growth rate, and tumor weight (H.J. Thompson, personal communication, July 13, 2009). Beginning at week 3, each rat was palpated along the mammary chains to detect any masses that may have

grown (Thompson, 2000). While masses were not expected by the third week, it was important to acclimate rats to this type of handling. Rats were palpated two times a week, and any masses (number and description of location) were recorded (Thompson, 2000). When the first mass appeared in a particular rat, this time point was recorded as the first incident of tumor in that rat. The number of rats with tumors was a measure of tumor incidence.

In addition, with each palpation, the number of masses was recorded to measure tumor multiplicity. At the end of the experiment, the tumors were removed and counted to make sure the numbers recorded by palpation were accurate (Thompson, 2000). After the rats were euthanized, the tumors were surgically removed from the rat and weighed on an analytical balance to determine the end weight of all tumors for each individual rat. Then the tumors were prepared for histopathology and all tumor measures were further confirmed with a histopathological diagnosis.

Behavioral Dependent Variables

Food Consumption

Food consumption was used as a measure of general health and to determine the effect of stress on the animal (Berger, 2009; Perry, 2009; Long, 2010, Hamilton, 2010; Starosciak, 2010). Stress can affect food consumption (Faraday, 2002; Levine & Morley, 1981; Grunberg & Straub, 1991; Berger, 2009; Perry, 2009; Long, 2010; Starosciak, 2010; Hamilton, 2010).

The rats had continuous access to food (Teklad 4% Mouse/Rat Diet 7001) and food consumption was measured two times every week. Food was

placed on the top of each cage lid. Food consumption was calculated by weighing each food tray with an electronic scale (Sartorious electronic scale), then subtracting that weight from the previously measured weight. When food was replenished, the new weight was recorded and used in the next calculation.

Open Field Activity (OF)

General activity, learning, depression, and anxiety or stress have all been measured using open field (Faraday, 2000; Shafer, 2006; Berger, 2009; Perry, 2009; Sarkisova, Kulikov, Midzyanovskaya, & Folomkina, 2008; Zhuang, Xu, & Chun-Zhi, 2007; Grippo, Beltz, & Johnson, 2003; Long, 2010; Hamilton, 2010; Starosciak, 2010). For the present experiment, the activity domains of interest were horizontal activity, vertical activity, and center time. Horizontal activity provides an assessment of general activity level as well as an index of depression, vertical activity (exploratory activity) was used as an index of depression, and center time was used as an index of anxiety.

Open field activity was measured after the MNU initiation phase as a baseline and then at the end of each week for a total of nine open field measures. Procedures for open-field activity were based on previous procedures performed in the Grunberg Laboratory (Faraday, 2000; Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Hamilton, 2010; Starosciak, 2010). Open-field activity were measured using an Omnitech/Accuscan Electronics Digiscan infrared photocell system (Test box model RXYZCM (16 TAO); Omnitech Electronics, Columbus, OH). Upon starting the measures, rats were individually placed in the center of a 40 x 40 x 30 cm clear Plexiglas chamber equipped with

infrared photo beams. A Plexiglas lid with ventilation holes was placed on top of the chambers to prevent rats from jumping out of the chambers. Horizontal activity was measured whenever an infrared beam was broken by the animal (infrared beams are located throughout the plane of the chamber floor) (Shafer, 2006; Berger, 2009; Perry, 2009; Long, 2010; Hamilton, 2010; Starosciak, 2010). Data were saved onto a computer by an Omnitech Model DCM-I-BBU analyzer. Once rats were placed in chambers and the chambers were switched on, the experimenter turned off the overhead lights and left while activity was monitored for one hour.

Ultrasonic Vocalizations (USV)

Ultrasonic vocalizations were measured once after the MNU initiation as a baseline and then one day at the end of each stress week for a total of nine USV measures throughout the experiment. Ultrasonic vocalizations were performed according to procedures previously performed in the Grunberg Laboratory (Long, 2010). Ultrasonic vocalizations were measured using a Med Associates, Inc. Ultrasonic Vocalization Detector (ANL-937-1, Med Associates, Inc., St. Albans, VT). Ultrasonic vocalizations were measured one rat at a time (only one rat was in the dedicated room at a time to make sure that vocalizations that were recorded were for a particular animal). Rats were placed in a clean rodent cage (46 cm x 36 cm x 20 cm) that had hardwood chip bedding (Pine-Dri). The Ultrasonic Vocalization Detector was placed on top of the cage lid, and was switched on. Ultrasonic vocalizations were recorded electronically for 2 minutes.

Data were collected and saved with Med PC IV software (Med Associates, Inc., St. Albans, VT).

MNU Preparation, Administration, and Disposal

MNU preparation and administration procedures were based on information obtained from Dr. H.J. Thompson, expert in this animal model of mammary cancer (Thompson, 2000; H.J. Thompson, personal communication, July 13, 2009). Prior to MNU purchase, the experimenter followed USUHS Environmental Health and Occupational Safety protocols for this particular chemical, which included purchasing appropriate personal protective equipment. The experimenter was fit-tested for an N-100 filtered mask prior to handling this chemical. In addition, the chemical fume hood in the Grunberg Laboratory was inspected to ensure that it was functioning properly. MNU was purchased from Ash Stevens (ASI-701), and stored in a freezer at -80° Celsius. The preparation of MNU was done in a chemical fume hood. The fume hood was lined with disposable bench paper to facilitate clean up. MNU was removed from the freezer, placed in a covered ice bucket, and transferred to the fume hood. MNU (224 mg as determined by that day's animal body weight measurements) was weighed directly into a glass injection vial via an analytical balance. The vial was stopped with a septum, wrapped in foil, and placed in an ice bucket. Prior to administration, 16 ml of 0.9% NaCl, acidified with a drop of acetic acid to achieve a pH of 4.06 (as tested by a pH meter) was added to the injection vial. The vial was secured with a septum and aluminum seal via crimper. The MNU was

dissolved in the saline solution by running the vial under warm tap water with brisk shaking (concentration of MNU was 14 mg/ml based on calculations).

On the day of administration, animals were weighed and the weights were recorded. In the Grunberg Laboratory, animals were administered MNU (50 mg MNU/kg body weight) by intraperitoneal injection with a 26 gauge, 3/8-inch sterile needle (Becton Dickinson, NJ).

After administration of MNU, all instruments that contained MNU or had contact with MNU were deactivated. MNU was deactivated based on procedures taught by Dr. Henry Thompson (December 4, 2009). Deactivation required using a quart sized heavy duty plastic container and mixing sodium carbonate and water until the sodium carbonate was oversaturated (e.g., continued to add sodium carbonate to water until sodium carbonate formed crystals and could no longer be dissolved completely in the water). This procedure took place inside the fume hood. All items that came into contact with MNU (e.g., needles, syringes, vial, spatula) were placed in this mixture for 24 hours. After 24 hours, EHS was contacted to pick up and dispose of the chemicals according to USUHS policy.

Procedure (See Table B at end of section for experimental timeline)

On the first day of the experiment, subjects were assigned to one of the six conditions. Upon randomization, rats were assigned identification numbers and tails were marked with a permanent marker to show identification. Cages were numbered corresponding to the animals in that cage and rat tails were coded with a marker using a stripe system that was commonly used in the

Grunberg Laboratory. Stripes placed on the base of the tail represented units of ten, and stripes placed on the ends of the tails represented units of ones. Tail markings occurred two times a week.

On the first three days, each subject was briefly gentled (by handling and talking around for about 3 minutes each) to attenuate or prevent stress responses from handling that would be required to measure body weight and to conduct other behavioral measures. On day two, body weight and food consumption were recorded and the subjects were injected with MNU. After administration of MNU, subjects were left in their home cages and only body weight, food consumption, and tail markings were performed the following week. On day 15, the subjects were acclimated to the locomotor chambers and ultrasonic vocalization cages.

Starting on day 7, food consumption, and body weight were measured and recorded two times a week for the duration of the experiment. Palpations began on day 14 and continued two times a week for the duration of the experiment. Any masses detected were recorded. Experimenters performing palpations were blind to treatment condition. Prior to palpations, experimenters performing palpations practiced on a model rat with masses and had an inter-rater reliability of 0.96 (intraclass correlation coefficient [ICC]) on mass measurements. On day 17, a baseline measure of ultrasonic vocalizations was taken, and on day 18, a baseline measure of locomotor activity was taken.

Starting on day 21, the stressors were introduced to the subjects that were assigned to the stress conditions three days a week, at various times within the

first several hours of their active period to make the stressor unpredictable for the subjects for the duration of the experiment. Subjects assigned to the no stress conditions remained in their housing room. After each stress/no stress exposure, the subjects then were exposed to music, noise, or no music/noise depending on their previously assigned condition for five hours. On the last two days of each week, behaviors of all subjects were measured in the locomotor chamber and in the ultrasonic vocalization chamber. There were a total of nine locomotor and ultrasonic vocalization measures throughout the experiment. All procedures occurred in the morning and afternoon (between 0100 hours and 1300 hours) throughout the experiment when the housing room was dark and rats were in their active phase of the day.

Table B. Experimental Timeline

Exp. Day	Age (days)	Procedures	DVs
1	20	Arrival, gentle, MT	
2	21	MNU, BW, FC	BW, FC
3	22	Gentle	
4	23		
7	26	BW, FC, MT	BW, FC
11	30	BW, FC, MT	BW, FC
14	33	BW, FC, MT, P	BW, FC, TL
15	34	Loco and USV accli	
17	36	USV baseline	USV
18	37	Loco baseline, P, BW, FC, MT	Loco, TL, BW, FC
21	41	BW, FC, MT, S/NS, M/N/NMN, P	BW, FC, TL
22	42	S/NS, M/N/NMN	
23	43	S/NS, M/N/NMN	
24	44	USV	USV
25	45	BW, FC, MT, Loco, P	BW, FC, Loco, TL
28	48	BW, FC, MT, S/NS, M/N/NMN, P	BW, FC, TL

29	49	S/NS, M/N/NMN	
30	50	S/NS, M/N/NMN	
31	51	USV	USV
32	52	BW, FC, MT, P, Loco	BW, FC, TL, Loco
35	55	BW, FC, MT, S/NS, M/N/NMN, P	BW, FC, TL
36	56	S/NS, M/N/NMN	
37	57	S/NS, M/N/NMN	
38	58	USV	USV
39	59	BW, FC, MT, Loco, P	BW, FC, Loco, TL
42	62	BW, FC, MT, P, S/NS, M/N/NMN	BW, FC, TL
43	63	S/NS, M/N/NMN	
44	64	S/NS, M/N/NMN	
45	65	USV	USV
46	66	BW, FC, MT, Loco, P	BW, FC, Loco, TL
49	69	BW, FC, MT, S/NS, M/N/NMN, P	BW, FC, TL
50	70	S/NS, M/N/NMN	
51	71	S/NS, M/N/NMN	
52	72	USV	USV
53	73	BW, FC, MT, P, Loco	BW, FC, Loco, TL
56	76	BW, FC, MT, S/NS, M/N/NMN, P	BW, FC, TL
57	77	S/NS, M/N/NMN	
58	78	S/NS, M/N/NMN	
59	79	USV	USV
60	80	BW, FC, P, Loco	BW, FC, TL, Loco
63	83	BW, FC, MT, S/NS, M/N/NMN, P	BW, FC, TL
64	84	S/NS, M/N/NMN	
65	85	S/NS, M/N/NMN	
66	86	USV	USV
67	87	BW, FC, P, Loco	BW, FC, TL, Loco
68	88	S/NS, M/N/NMN	
69	89	S/NS, M/N/NMN	
70	90	S/NS, M/N/NMN, BW, FC, MT, P	BW, FC, TL
71	91	USV	USV

72	92	BW, FC, P, Loco	BW, FC, TL, Loco
74	94	Euthanasia	TW, TM

BW = Body weight; FC = Food consumption; MT = Mark tails; Loco = Locomotor; USV = Ultrasonic vocalizations; P = Palpations; TL = Tumor log; TW = Tissue weights; TM = Tumor Measurements; S/NS = Stress/No stress exposure; M/N/NMN = Music, noise, no music/noise exposure; Accli = acclimation

Data Analytic Strategy

Subjects were randomly assigned to housing conditions upon arrival. Analyses of variance (ANOVA) were used for the majority of the data analyses. All behavioral measures were analyzed to determine if subjects in different conditions were initially (baseline or first measure) significantly different. If there were significant differences at initial behavioral measurement, then these initial/baseline data were used as covariates. Additionally, in analyses using repeated-measures ANOVAs, if there were violations of the assumption of sphericity, the Greenhouse-Geisser correction was used (indicated by adjusted degrees of freedom). However, it is important to note that all analyses were not ANOVAs because of the unexpected number of animals that developed tumors versus animals that did not develop tumors (it was expected that most of the animals would develop tumors, but only 40 animals developed tumors). In addition, it is important to note that all analyses involving tumor measures or taking tumor presence into account had tumors that were confirmed as malignant mammary carcinomas from a veterinary pathologist from Histoserv (Germantown, MD).

Body weight and food consumption were analyzed using two-way repeated-measures ANOVAs (stress condition x sound condition) to assess changes over time throughout the experiment. Open-field activity was analyzed using two-way repeated-measures ANOVAs (stress condition x sound condition) to examine the effects of stress and music/noise on center time activity, an index of anxiety, vertical activity and horizontal activity (indices of depression). All open-field analyses had time as the within-subject factor and stress condition and sound condition as the between-subjects factors. Ultrasonic vocalizations were analyzed using two-way repeated-measures ANOVAs (stress condition x sound condition) to examine the effects of stress and music/noise on an index of positive and negative affect. All ultrasonic vocalization analyses had time as the within-subject factor and stress condition and sound condition as the between-subjects factors. Because tumor progression occurs over time, ANOVAs were performed on body weight, food consumption, locomotor activity, and ultrasonic vocalizations at the last measure to determine if there were significant differences.

Serum corticosterone was analyzed using a two-way ANOVA to examine the effects of stress and music/noise on the stress response. This analysis also had stress condition and sound condition as the between-subjects factors. Tumor weight analyses included only animals with tumors. Because homogeneity of variance was not violated, a natural log transformation was conducted and allowed for a normal distribution in which a two-way ANOVA was conducted with the stress condition and sound condition serving as between-

subjects factors. Spleen weight and adrenal gland weight were analyzed using a two-way ANOVA to examine the effects of stress and music/noise on end tissue weights. This analysis had stress condition and sound condition as the between-subjects factors.

With these analyses, only significant results were examined with Tukey's HSD *post-hoc* tests (Keppel, 1991) to determine where the significant differences occurred. In addition to these subsequent analyses, exploratory analyses were performed consisting of performing a split-file using presence of tumor(s) to determine if results were different based on tumor status.

Time of first tumor was analyzed via a survival analysis. Survival analysis involves examining the distribution of time to event variables. The Kaplan Meier procedure was used. Kaplan Meier curves were used to determine significant differences between each group (e.g., stress condition and sound condition) at predicting status of tumor occurrence. An overall test of equality of survival times, Log Rank Chi-Square, was used to determine differences between curves.

Tumor multiplicity was analyzed using a Kruskal-Wallis test. A nonparametric test was used to examine if there was a stress or sound effect rather than a two-way ANOVA because the data violated assumptions of ANOVA (e.g., the residuals were not a normal distribution and homogeneity of variance was violated). To determine if there was a significant difference between groups on tumor growth rates, first slopes were calculated to determine the average growth rates of tumor for each animal. When all animals were included in the analysis, a Kruskal-Wallis test was performed because the data violated the

assumptions of ANOVA (e.g., the residuals were not a normal distribution and homogeneity of variance was violated). When only animals with tumors were included in the analysis, homogeneity of variance was not violated and a natural log transformation was conducted and allowed for a normal distribution in which a two-way ANOVA was conducted with the stress condition and sound condition serving as between-subjects factors. Tumor incidence, or the number of animals with tumor presence, was analyzed separately based on independent variables (e.g., a Chi-Square was performed based on stress condition and a separate Chi-Square was performed based on sound condition). To determine if there was an interaction between stress condition and sound condition on tumor incidence, a Binary Logistic Regression was performed.

Some data were excluded from analyses because of early animal death. Prior to behavioral measures or experimental manipulation, one animal died, and therefore, was not included in any analyses. Because of some early animal death, four data points of 890 total data points (0.45%) were excluded from the body weight data set; four data points of 890 total data points (0.45%) were excluded from the food consumption data set; three data points of 801 total data points (0.37%) were excluded from the low ultrasonic vocalizations data set; three data points of 801 total data points (0.37%) were excluded from the high ultrasonic vocalizations data set; four data points of 801 total data points (0.50%) were excluded from the locomotor center time/total time data set; four data points of 801 total data points (0.50%) were excluded from the locomotor horizontal

activity data set; and four data points of 801 total data points (0.50%) were excluded from the locomotor vertical activity data set.

Results

The results are presented by dependent variable. Only significant and interesting trends are presented in text with corresponding figures. To see all statistical results refer to Appendix D.

Body Weight

Animals began at approximately the same body weight. There was a significant main effect for time (see Figure 1), indicating that all animals gained weight over the course of the experiment ($F [1.767, 143.155] = 4711.696$, $p < 0.001$). Because only 40 animals developed tumors throughout the experiment a split-file was performed to determine if there were any significant differences in animals that developed tumors and in animals that did not develop tumors.

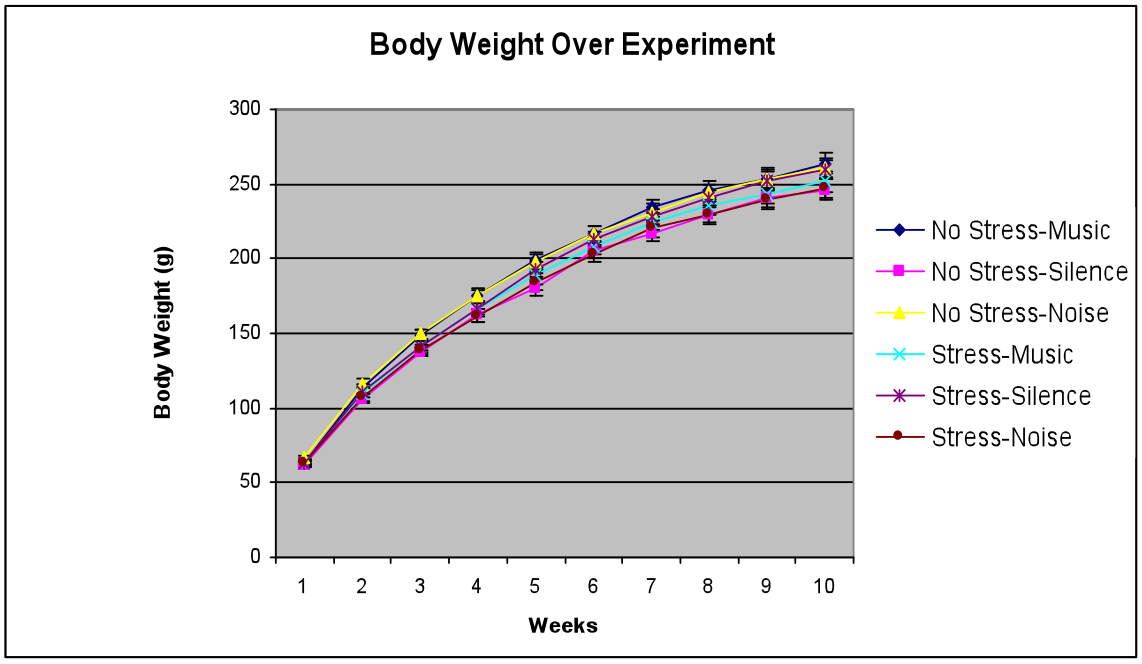

Figure 1. Mean Body Weight Over Experiment

In animals with tumors (see Figure 2), there was a significant main effect for stress where animals in the no stress condition gained more weight than did animals in the stress condition ($F [1, 32] = 4.251$, $p < 0.05$). Animals with tumors had a significant stress by sound interaction; in the non-stressed condition, animals in the silence condition gained less weight than did animals in the music and noise conditions; in the stressed condition, animals in the silence condition gained more weight than did animals in the music and noise conditions ($F [2, 32] = 6.746$, $p < 0.01$). There also was a significant time by stress by sound interaction in animals with tumors but no clear pattern emerged ($F [4.489, 71.817] = 3.638$, $p < 0.01$). In animals without tumors there was only a significant main effect for time where all animals gained weight over time ($F [1.411, 60.652] = 2643.276$, $p < 0.001$).

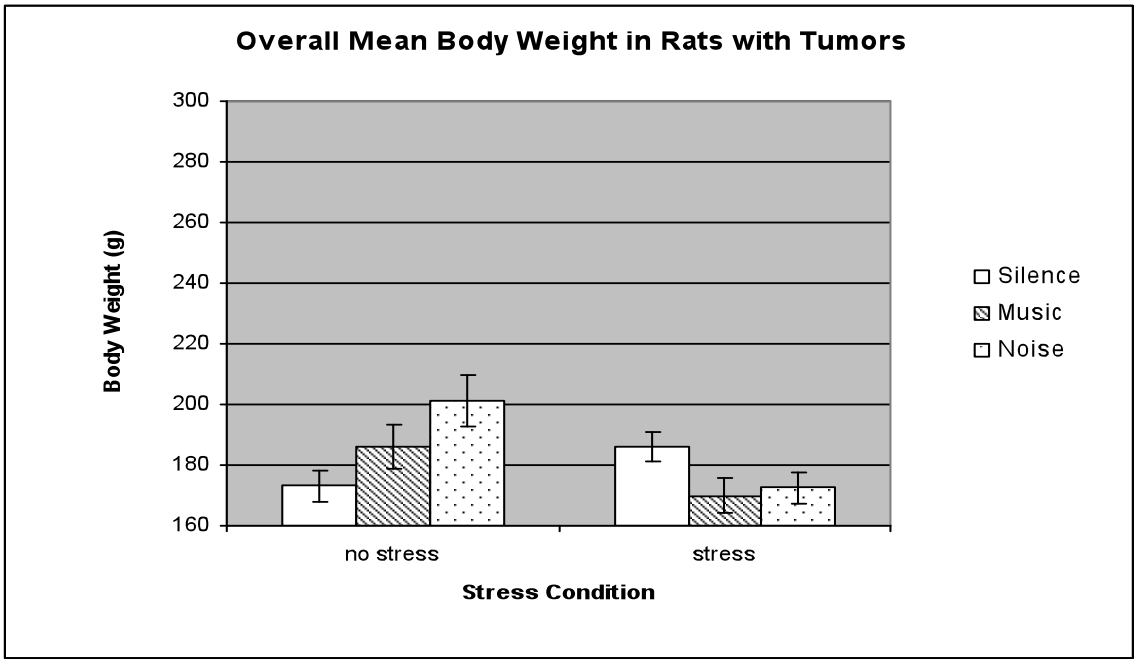

Figure 2. Overall Mean Body Weight in Rats with Tumors

The last body weight measure was analyzed to determine if there were differences at the end of the experiment. There were no significant differences in body weight among conditions. A split-file was performed to determine if there were differences depending on tumor status. In animals with tumors (see Figure 3), there was a significant stress by sound interaction. In the non-stressed condition, animals in the silence condition gained less weight than did animals in the music and noise conditions; in the stress condition, animals in the silence condition gained more weight than did animals in the music and noise conditions ($F [2, 32] = 6.545$, $p < 0.01$). There were no significant differences in animals without tumors.

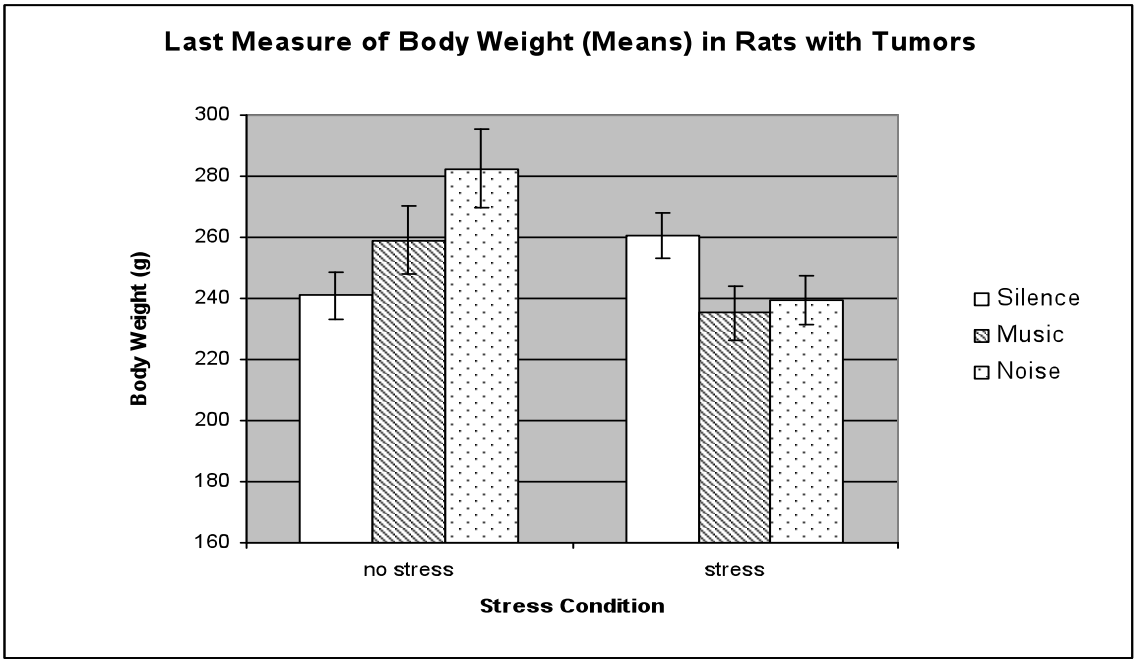

Figure 3. Last Measure of Body Weight (Means) in Rats with Tumors

In summary, all animals gained weight over the course of the experiment. Animals with tumors had a stress by sound interaction where the non-stressed rats gained less weight in the silence condition compared to the music and noise conditions, whereas the stressed rats gained more weight in the silence condition than in the music and noise conditions.

Food Consumption

There were initial (first food consumption measure before experimental manipulations) differences in food consumption between conditions. There was a significant main effect for stress where animals that were going to be stressed consumed more food than did animals that were not going to be stressed condition ($F [1, 83] = 12.336$, $p = 0.001$). There was a significant main effect for sound where animals that were going to be exposed to silence consumed less

food than did animals that were going to be exposed to music (F [2, 83] = 6.175, p < 0.01). There was a significant stress by sound interaction where animals that were going to be exposed to silence consumed more food in the condition that was going to be stressed compared to the condition that was not going to be stressed (F [2, 83] = 5.463, p < 0.01). Because of these initial differences, the first food consumption measure was used as a covariate in subsequent food consumption analyses.

There was a significant main effect for time (see Figure 4) where all animals increased food consumption throughout the course of the experiment (F [2.294, 183.497] = 4.014, p < 0.05).

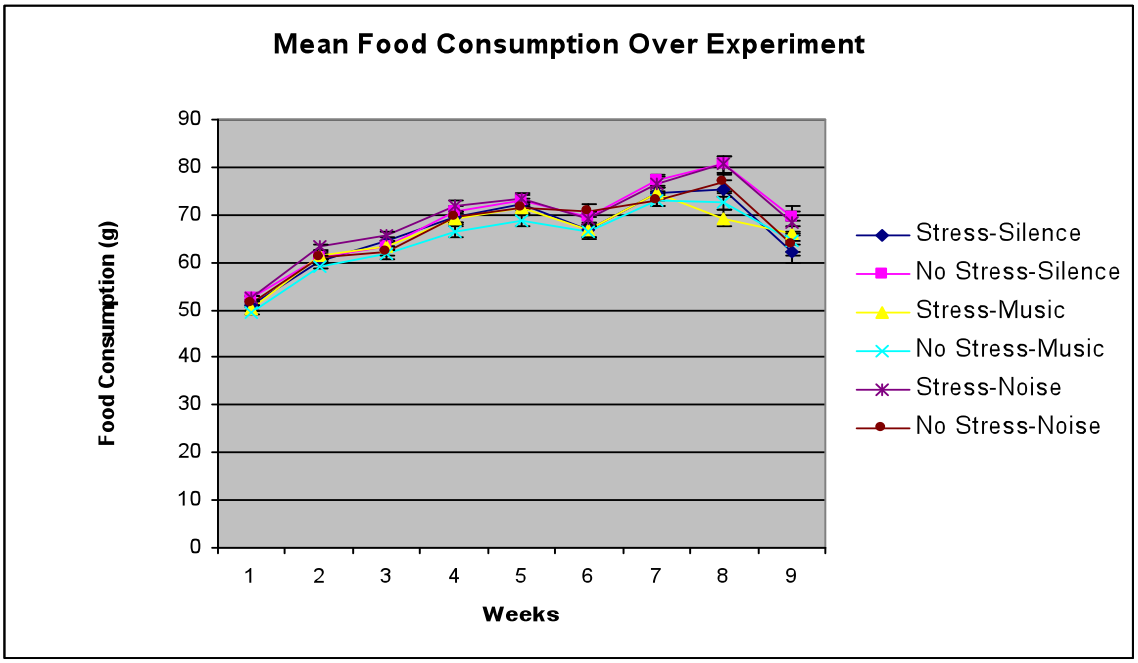

Figure 4. Mean Food Consumption Over Experiment

There was a significant main effect for sound (see Figure 5) where the music condition consumed less food than did the silence and noise conditions

(F [2, 80] = 3.447, p < 0.05). There was a significant time by sound interaction where the music group increased food consumption at a slower rate than did the silence and noise conditions (F [4.587, 183.497] = 3.235, p = 0.01).

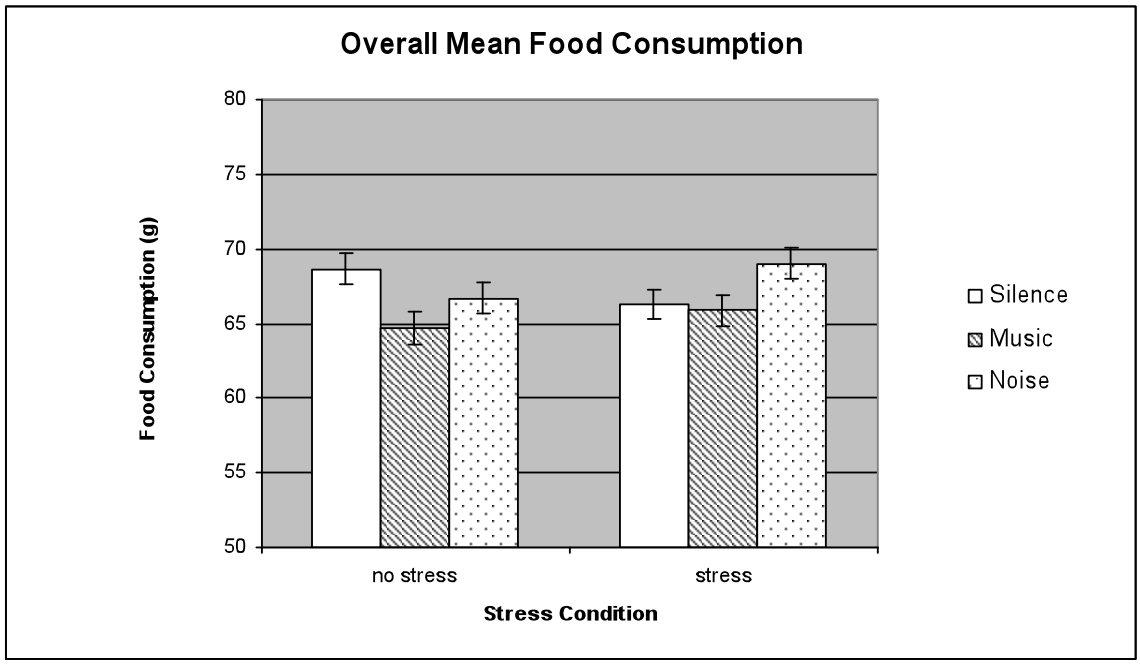

Figure 5. Overall Mean Food Consumption (Means are averaged across weekly food consumption over the entire experiment)

A split-file was performed based on tumor status. There were no significant differences found in animals with tumors. In animals without tumors (see Figure 6) there was a significant main effect for sound where the music condition consumed lower amounts of food than did the noise condition (F [2, 42] = 4.052, p < 0.05). There also was a significant stress by sound interaction where the non-stressed silence condition consumed greater amounts of food than did the non-stressed music and noise conditions, but the stressed silence condition consumed less food than did the stressed music and noise conditions (F [2, 42] = 8.258, p = 0.001).

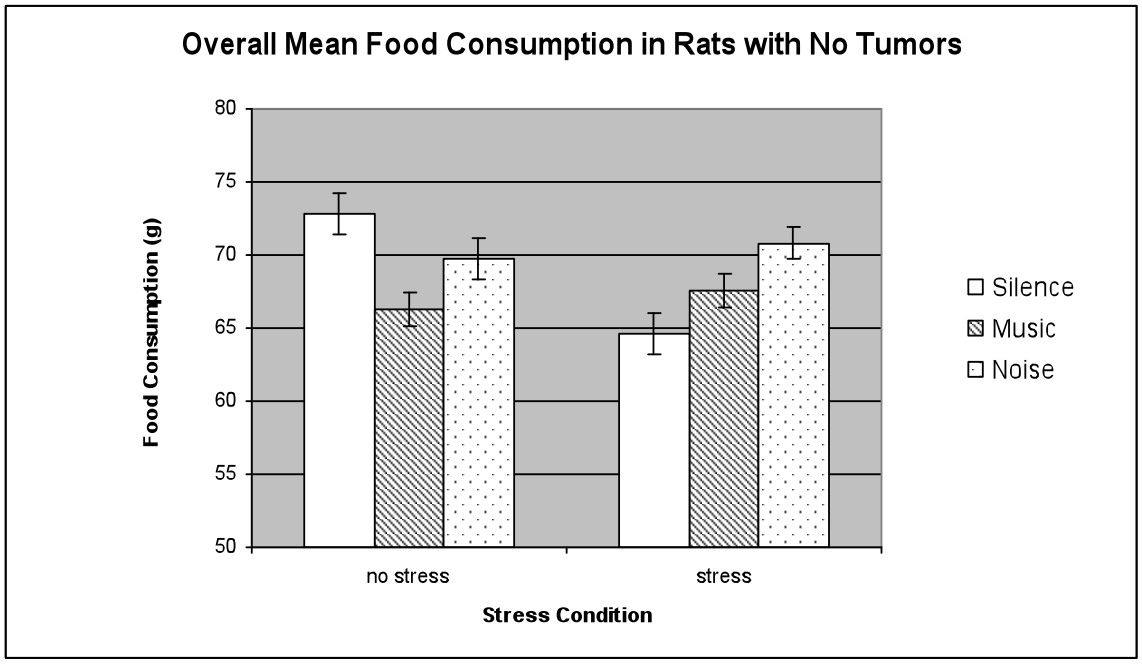

Figure 6. Overall Mean Food Consumption in Rats with No Tumors

The last measure of food consumption was analyzed to determine if there were any significant differences between conditions at the end of the experiment. There was a significant stress by sound interaction (see Figure 7) where the non-stressed silence condition consumed more food than did the non-stressed music and noise conditions, but the stressed silence condition consumed less food than did the stressed music and noise conditions ($F [2, 80] = 3.502$, $p < 0.05$).

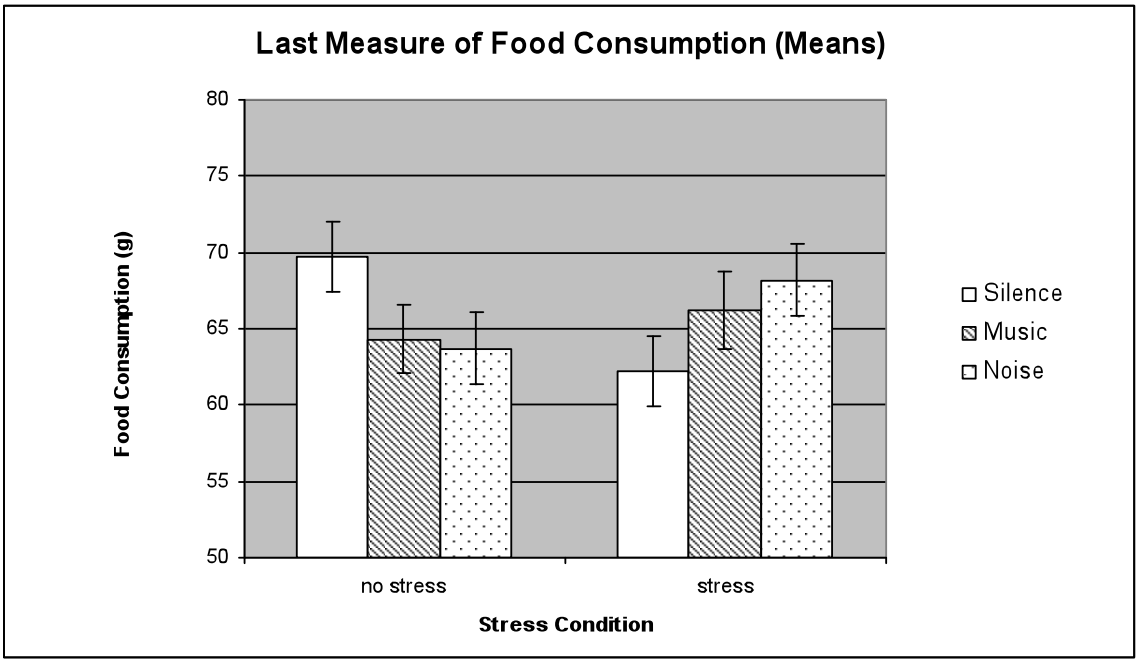

Figure 7. Last Measure of Food Consumption (Means)

A split-file was performed based on tumor status. There were no significant differences in food consumption in animals with tumors. In animals without tumors (see Figure 8), there was a significant stress by sound interaction where the non-stressed silence condition consumed more food than did the non-stressed music and noise conditions, but the stressed silence condition consumed less food than did the stressed music and noise conditions ($F [2, 42] = 5.535$, $p < 0.01$).

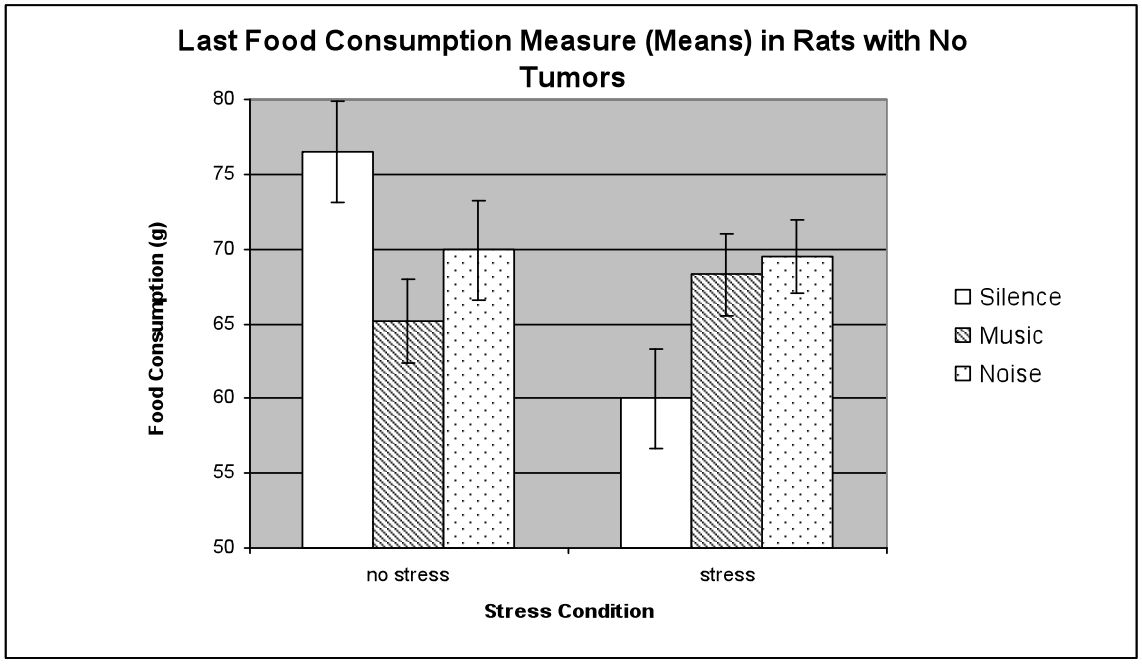

Figure 8. Last Food Consumption Measure (Means) in Rats with No Tumors

In summary, all animals had a gradual increase in food consumption over the time of the experiment. Overall, the music condition increased food consumption at a slower rate than did the silence and noise conditions. For animals without tumors: when not exposed to stress, the animals consumed more food in the silence than in the other sound conditions, but when exposed to stress, animals in the silence condition consumed less food than in the other sound conditions. For animals with tumors, there were no significant differences in food consumption among conditions.

Stress Measures

Serum Corticosterone. There was a significant main effect for sound (see Figure 9) where the noise condition had significantly lower levels of serum corticosterone than did the silence and music conditions ($F [2, 83] = 5.806$, $p <$

0.01). There was a significant stress by sound interaction where there was a stress effect for the silence and music conditions with more serum corticosterone when stressed; however, in the non-stressed noise condition had higher serum corticosterone than did the stressed noise condition (F [2, 83] = 3.727, p < 0.05).

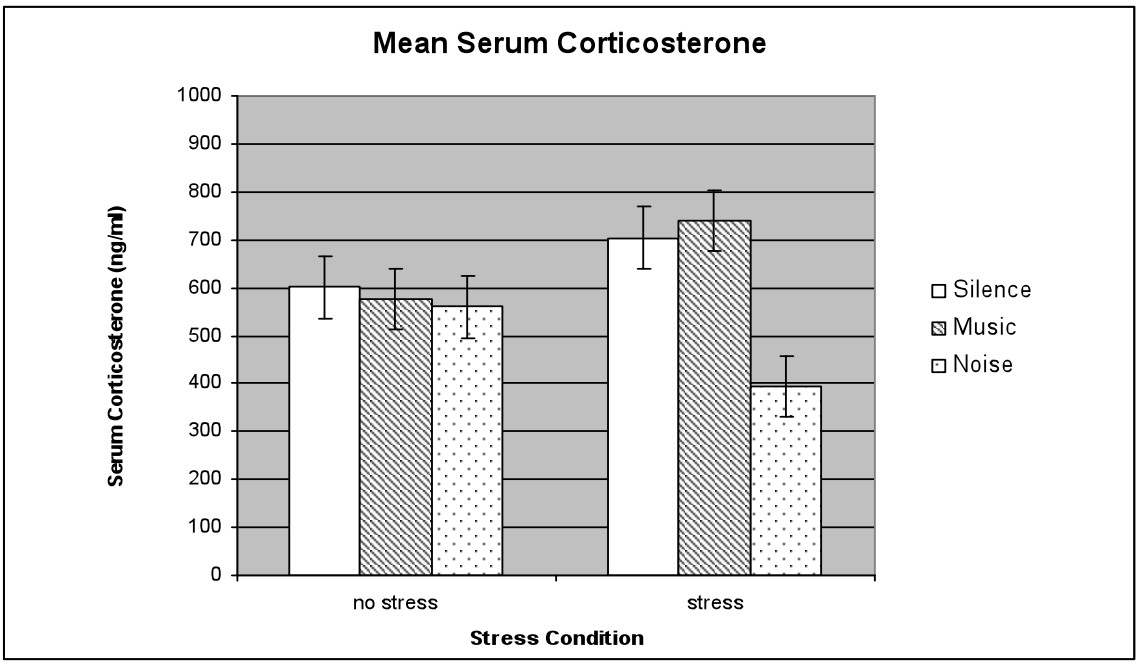

Figure 9. Mean Serum Corticosterone

When a split-file was conducted, these differences were only found in animals without tumors (see Figure 10). In animals without tumors there was a significant main effect for sound where the noise condition had lower levels of serum corticosterone than did the silence and music conditions (F [2, 43] = 7.215, p < 0.01). However, this effect is better explained by the significant stress by sound interaction where the stressed silence and music conditions had higher levels of serum corticosterone than did the non-stressed silence and music conditions, but the stressed noise condition had lower levels of serum

corticosterone than did the non-stressed noise condition ($F [2, 43] = 6.943$, $p < 0.01$).

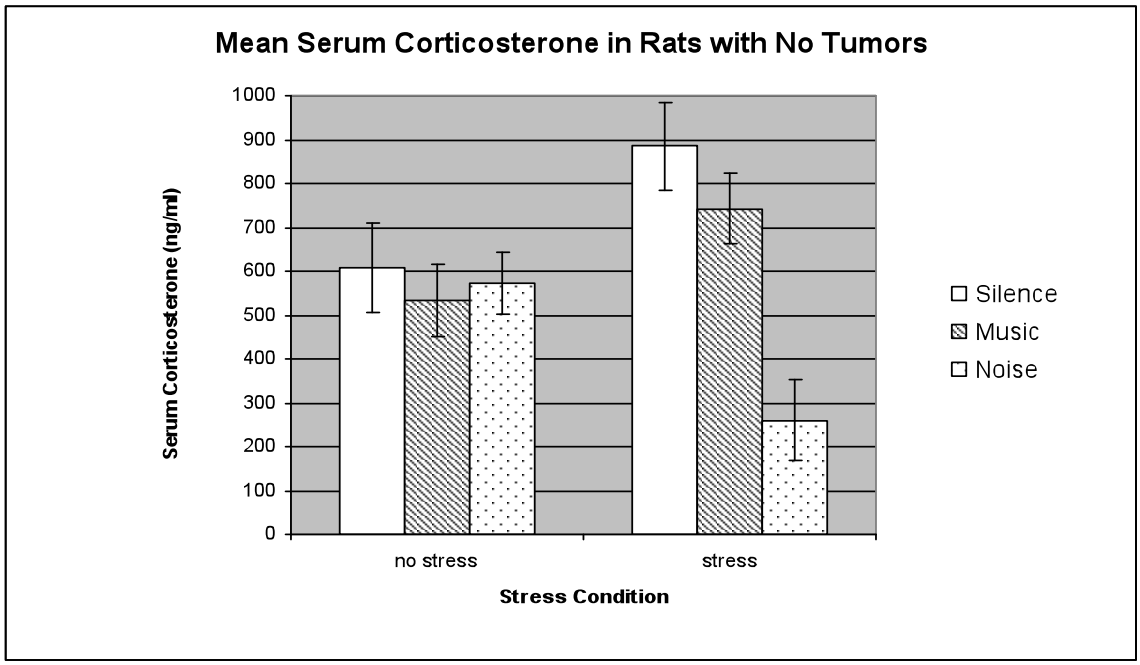

Figure 10. Mean Serum Corticosterone in Rats with No Tumors

In summary, there is a significant stress by sound interaction in the measure serum corticosterone where there appears to be a stress effect in the silence and music conditions with higher levels in the stressed condition and lower levels in the unstressed condition. However, the reverse occurs in the noise condition where serum corticosterone levels are higher in the unstressed condition and lower in the stressed condition. These effects appear to be due to the animals without tumors, as there were no significant differences in serum corticosterone in animals with tumors.

Spleen Weight. There were no significant differences among conditions for spleen weight.

Adrenal Glands Weight. There were no significant differences among conditions for adrenal glands weight.

Tumor Measures

Tumor Incidence. There were no statistically significant differences between conditions for tumor incidence because there was not enough statistical power. However, there are many patterns that are worth noting (see Figure 11). In the non-stressed condition, the silence condition had a greater percentage of animals that develop tumors (57%), the music condition had a lower percentage (40%), and the noise condition had the least (20%). In the stress condition, there is a similar pattern where the silence condition had a 60% incidence and the music condition had a 40% incidence. However, the noise condition when exposed to stress had an incidence of 53%. With regard to the noise condition, stress may increase the likelihood of tumor development.

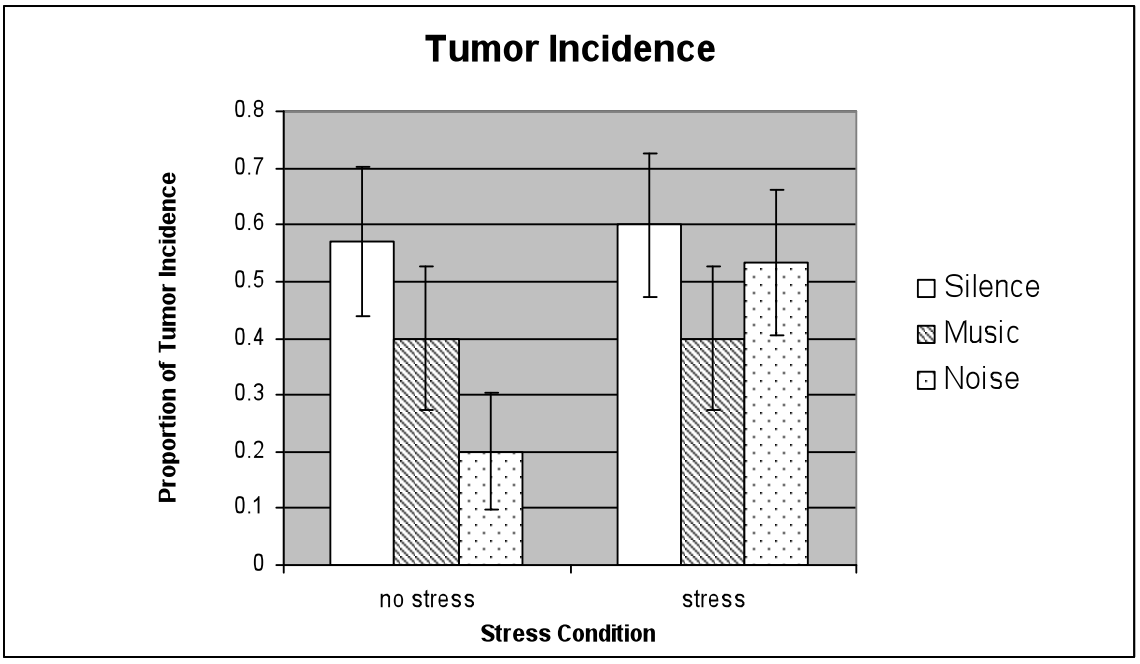

Figure 11. Tumor Incidence (No stress: Silence n = 8, Music n = 6, Noise n = 3; Stress: Silence n = 9, Music n = 6, Noise n = 8)

Tumor Multiplicity. There were no significant differences between conditions in tumor multiplicity because of lack of power. Again, an interesting pattern appeared that is worth noting (see Figure 12). Because the data were analyzed using the Kruskal-Wallis Test the results are presented as mean ranks (with number of tumors per subject ranked in ascending order from 1 to 89). In the non-stressed condition, the silence condition had a tumor multiplicity mean rank of approximately 51, the music condition had a mean rank of approximately 44, and the noise condition had the lowest mean rank of approximately 32. In the stressed condition, the silence condition had a mean rank of approximately 48, the music condition had a mean rank of approximately 46, and the noise condition had a mean rank of approximately 49. The condition with the lowest

tumor multiplicity was the noise condition that was not exposed to stress. Stress may increase the number of tumors that develop in the noise condition.

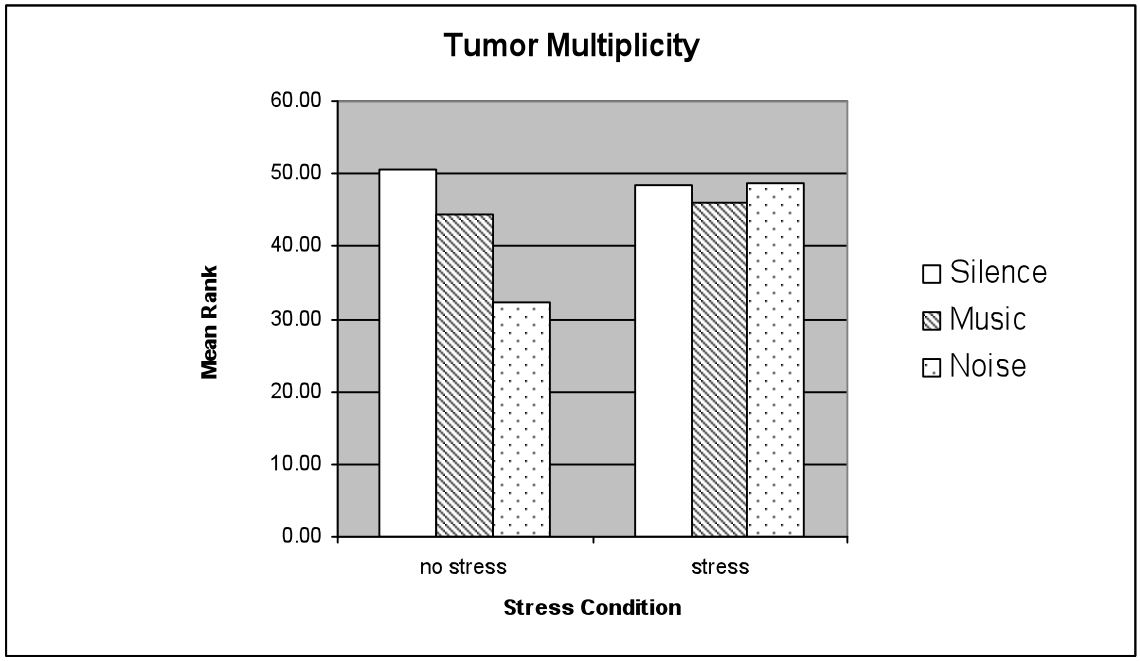

Figure 12. Tumor Multiplicity

Time to Event. There were no significant differences between conditions when first tumor detection occurred.

Tumor Growth. There were no significant differences between conditions in tumor growth rate. An interesting pattern emerged that is worth noting. Because the data were analyzed using the Kruskal-Wallis Test, the results are presented as mean ranks. In the non-stressed condition, the silence condition had a tumor growth mean rank of approximately 52, the music condition had a mean rank of approximately 45, and the noise condition had the lowest mean rank of approximately 32. In the stressed condition, the silence condition had a mean rank of approximately 49, the music condition had a mean rank of approximately

45, and the noise condition had a mean rank of approximately 47. It appears that tumor growth was slower in animals in the noise condition that were not exposed to stress.

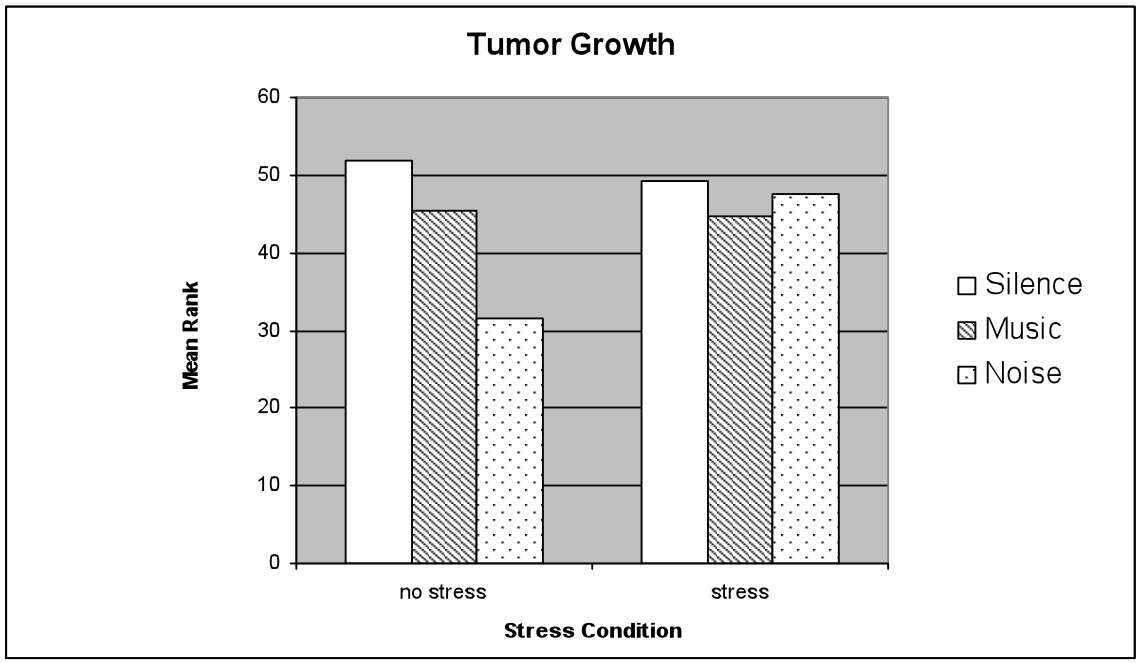

Figure 13. Tumor Growth

Tumor Weight. There were no significant differences between conditions in end tumor weight.

To summarize the results with regard to the tumor measures, there were no statistically significant findings. However, there was a notable trend in tumor incidence, tumor multiplicity, and tumor growth where the non-stressed noise condition had lower tumor incidence, tumor multiplicity, and tumor growth compared to all other conditions. It appears that stress may increase tumor incidence, tumor multiplicity, and tumor growth in the noise condition. A point biserial correlation was performed to determine if the tumor measures (tumor

growth, time to event, tumor multiplicity, tumor incidence, and tumor weight) were correlated because of this consistent trend. All tumor measures were significantly correlated ($p < 0.01$) with correlations ranging between 0.518 – 0.954.

Open Field Locomotor Activity (General Activity and Learning)

Horizontal Activity (General Activity). Horizontal activity is a measure of general activity and health. It is also an index of depression (i.e., increased horizontal activity indicates more exploration and interest suggesting less depressive-like behavior). Rather than repeating the same results, please refer to the horizontal activity results located under "Depression-like Behavior."

Within-Session Activity (Simple Learning). Because of the number of within-session activity runs and lack of an overall pattern among the runs, the following section only includes the interpretable results for each within-session activity run. To see a complete write-up of results found for all within-session activity runs refer to Appendix E.

Baseline Within-Session (before stress or sound manipulations). At baseline, activity decreased over time in all conditions ($F [5.965, 495.063] = 170.775$, $p < 0.001$) (see Figure 14).

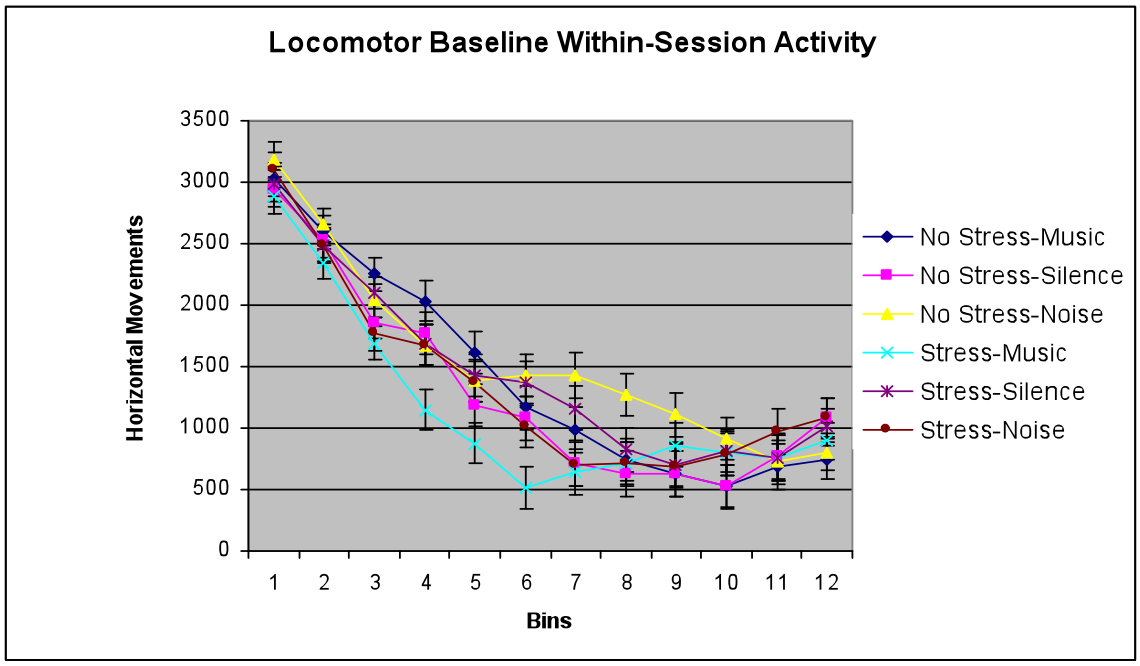

Figure 14. Locomotor Baseline Within-Session Activity

A split-file by tumor status was performed. In animals without tumors, activity decreased over time ($F [5.598, 240.734] = 75.803$, $p < 0.001$). In animals with tumors, activity decreased over time ($F [5.510, 187.340] = 75.857$, $p < 0.001$). In animals with tumors, there was an effect for sound where the noise condition had greater amounts of activity than did the silence condition, and the silence condition had more activity than did the music condition (noise > silence > music) ($F [2, 34] = 4.841$, $p < 0.05$).

In summary, animals habituated (simple learning) to the locomotor arena. In animals with tumors the music condition learned faster than did the silence condition, and the music and silence conditions learned faster than did the noise condition.

Run 1 Within-Session Activity (first week of stress and sound manipulations). In Run 1, all conditions decreased activity over time (F [6.652, 552.138] = 211.098, $p < 0.001$) (see Figure 15). There was a significant time by sound interaction where the silence condition decreased activity at a slower rate than did the music and noise conditions (F [13.305, 552.138] = 2.823, $p = 0.001$). There was a significant stress by sound interaction where the non-stressed music condition had more activity than did the stressed music condition (F [2, 83] = 3.380, $p < 0.05$).

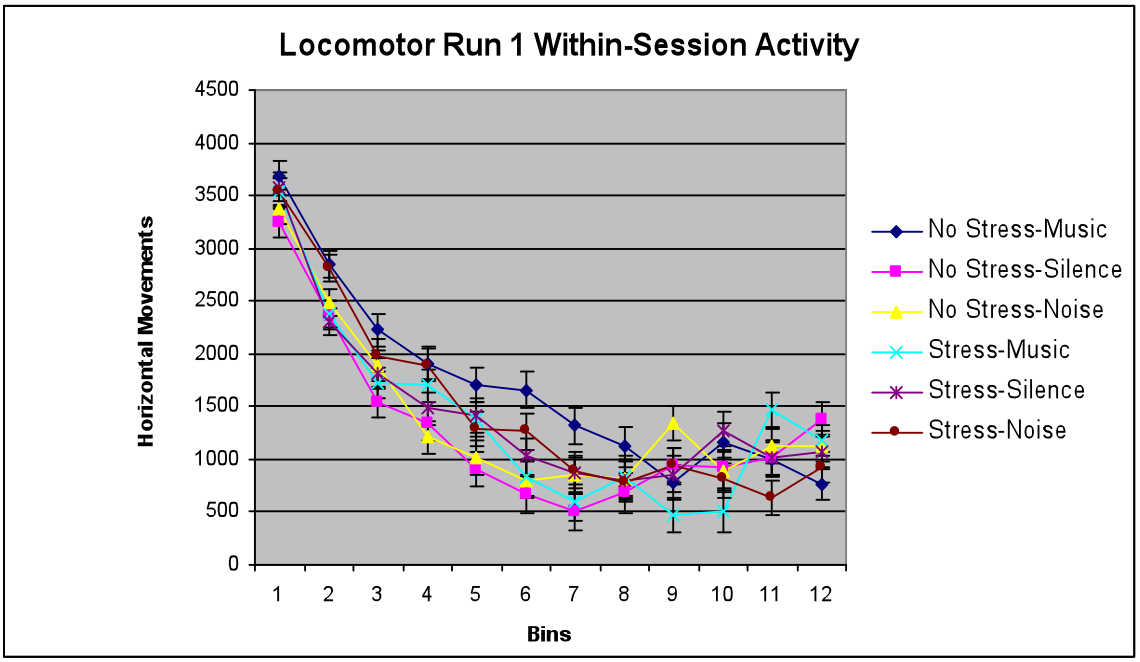

Figure 15. Locomotor Run 1 Within-Session Activity

A split-file by tumor status was performed. In rats with tumors, activity decreased over time (F [6.348, 215.820] = 82.516, $p < 0.001$). In animals without tumors, activity decreased over time in all conditions (F [5.972, 256.777] = 105.789, $p < 0.001$). There was a significant stress by sound interaction where

non-stressed music condition had more activity than did the stressed music condition, and the stressed silence condition had more activity than did the non-stressed silence condition ($F [2, 43] = 4.769$, $p < 0.05$).

In summary, all animals habituated to the locomotor arena. The silence condition appeared to habituate slower than did the music and noise conditions. In the music condition, the non-stressed learned faster than did the stressed; however, this effect may be due to the animals without tumors. When not stressed, the silence condition learned fastest, followed by the noise condition, and then the music condition.

Run 2 Within-Session Activity (second week during stress and sound manipulations). At Run 2, all animals decreased within-session activity over time ($F [6.879, 570.954] = 263.664$, $p < 0.001$) (see Figure 16).

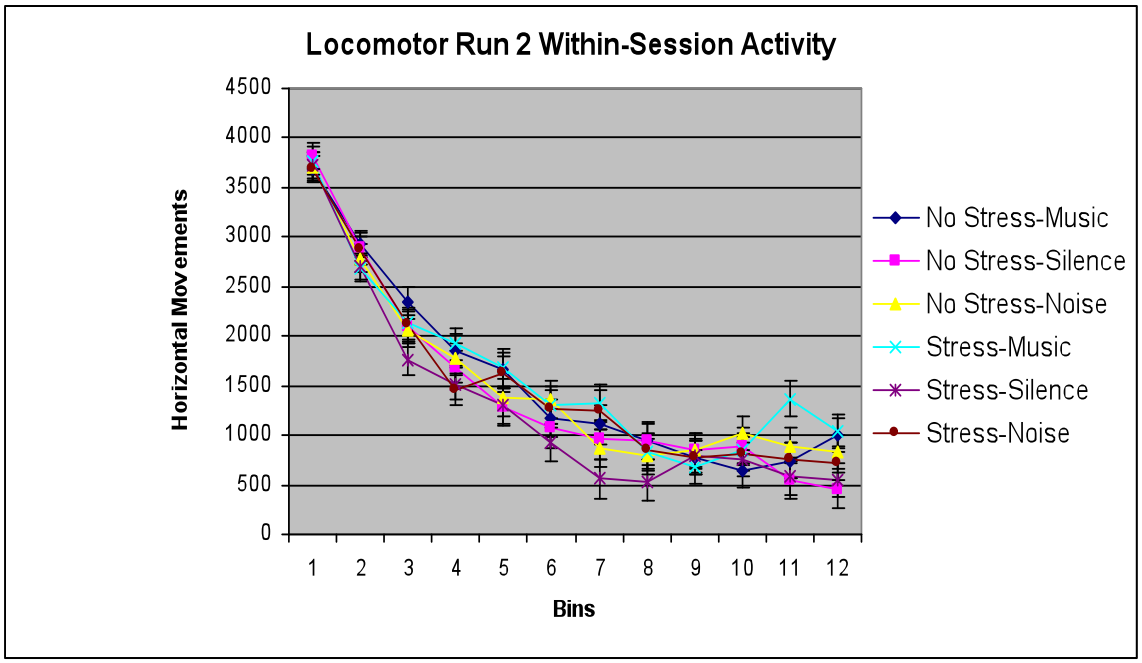

Figure 16. Locomotor Run 2 Within-Session Activity

A split-file was performed based on tumor status. In animals without tumors, activity decreased over time ($F [6.217, 267.323] = 134.813$, $p < 0.001$). In animals with tumors, activity decreased over time ($F [6.370, 216.583] = 100.444$, $p < 0.001$). There was an effect for stress where non-stressed animals had greater activity than did the stressed animals ($F [1, 34] = 4.270$, $p < 0.05$). There was an effect for sound where the noise condition had greater activity than did the music condition, and the noise and music conditions had greater activity than the silence condition ($F [2, 34] = 6.515$, $p < 0.01$). There also was a stress by sound interaction where the non-stressed noise condition had greater activity than did the stressed noise condition ($F [2, 34] = 4.274$, $p < 0.05$).

In summary, all animals habituated to the locomotor arena over time. In animals with tumors stress increased learning more than did no stress, and this effect is especially apparent in animals in the noise condition. Further, the silence condition learned the fastest, followed by the music condition, and then the noise condition.

Run 3 Within-Session Activity (third week of stress and sound manipulations). At Run 3, all animals decreased activity over time ($F [7.327, 608.149] = 229.614$, $p < 0.001$) (see Figure 17).

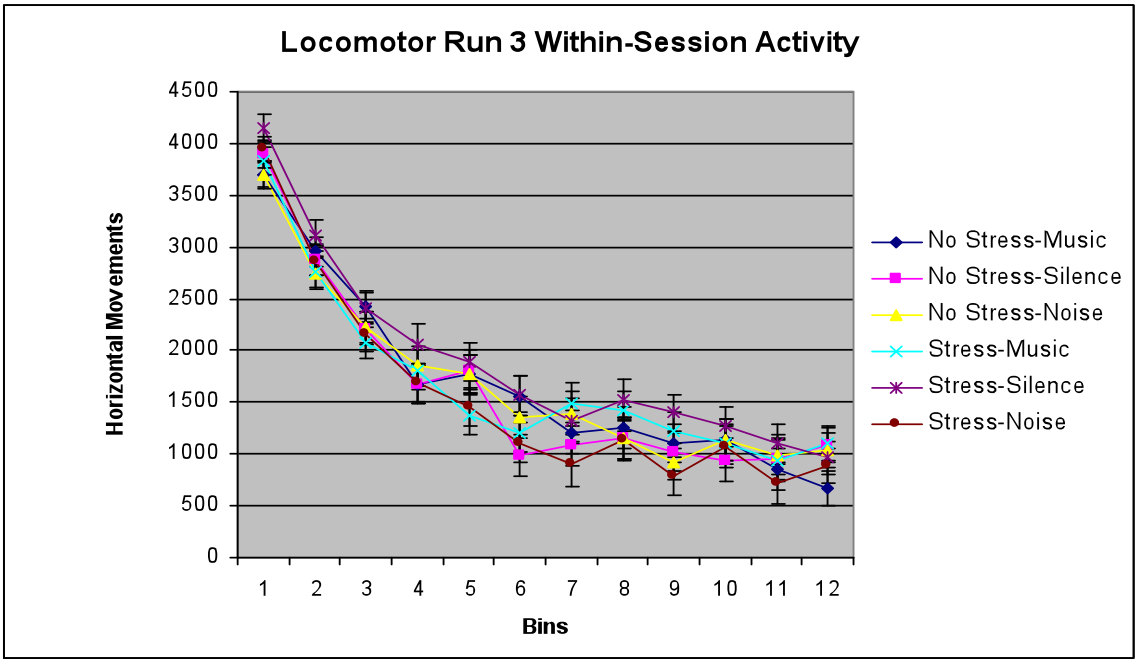

Figure 17. Locomotor Run 3 Within-Session Activity

A split-file was performed based on tumor status. In animals without tumors, activity decreased over time in all conditions ($F [6.292, 270.561] = 117.659$, $p < 0.001$). In animals with tumors, activity decreased over time ($F [6.600, 224.395] = 88.174$, $p < 0.001$). There was a stress by sound interaction where the non-stressed music condition had less activity than did the stressed music condition, and the non-stressed noise condition had more activity than did the stressed noise condition ($F [2, 34] = 3.307$, $p < 0.05$).

In summary, all animals habituated to the locomotor arena over time. In animals with tumors, the non-stressed music condition learned faster than the stressed music condition, but the stressed noise condition learned faster than the non-stressed noise condition.

Run 4 Within-Session Activity (fourth week of stress and sound manipulations). At Run 4, activity declined over time in all conditions (F [7.754, 643.558] = 252.599, $p < 0.001$) (see Figure 18). There was a time by stress interaction where the stressed condition had a faster decrease in activity than did the non-stressed condition (F [7.754, 643.558] = 2.191, $p < 0.05$).

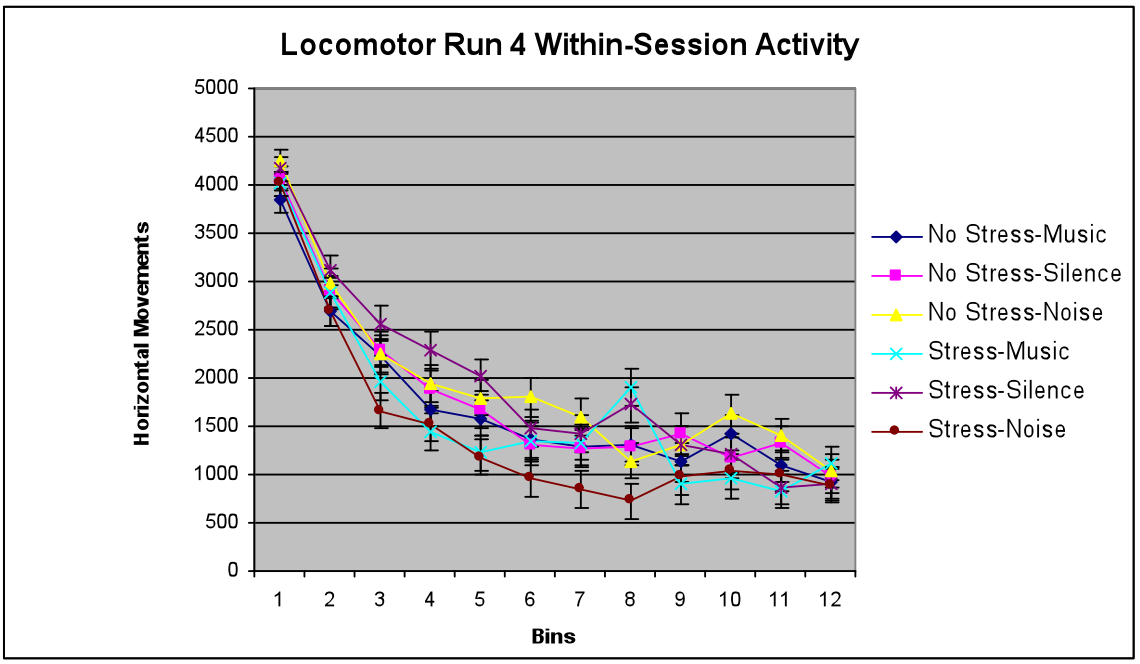

Figure 18. Locomotor Run 4 Within-Session Activity

A split-file was performed based on tumor status. In animals without tumors, activity decreased over time (F [7.263, 312.299] = 122.059, $p < 0.001$). In animals with tumors, activity decreased over time (F [6.731, 228.868] = 101.246, $p < 0.001$). There was a time by stress interaction where the stressed condition decreased activity at a faster rate than did the non-stressed condition (F [6.731, 228.868] = 2.364, $p < 0.05$). There was a stress by sound interaction

where the stressed noise condition had lower activity than did the non-stressed noise condition (F [2, 34] = 7.187, p < 0.01).

In summary, all animals habituated to the locomotor arena. The stress condition learned faster than the non-stressed condition, especially in the noise condition. These stress effects may be due to the animals with tumors.

Run 5 Within-Session Activity (fifth week of stress and sound manipulations). At Run 5, activity decreased over time in all conditions (F [8.408, 697.892] = 248.637, p < 0.001) (see Figure 19).

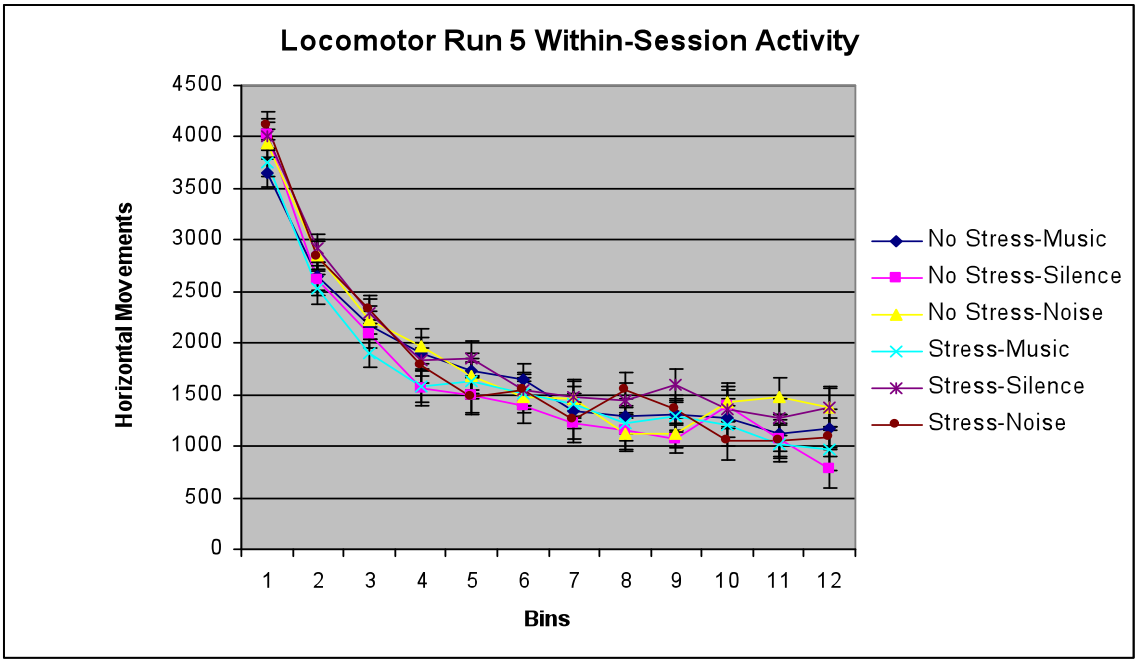

Figure 19. Locomotor Run 5 Within-Session Activity

A split-file was performed based on tumor status. Activity decreased over time in both animals with tumors (F [7.416, 252.140] = 90.961, p < 0.001) and animals without tumors (F [8.147, 350.322] = 136.203, p < 0.001). In summary, all animals habituated to the locomotor arena over the session.

Run 6 Within-Session Activity (sixth week of stress and sound manipulations). At Run 6, activity decreased over time in all conditions (F [7.713, 632.501] = 230.977, p < 0.001) (see Figure 20).

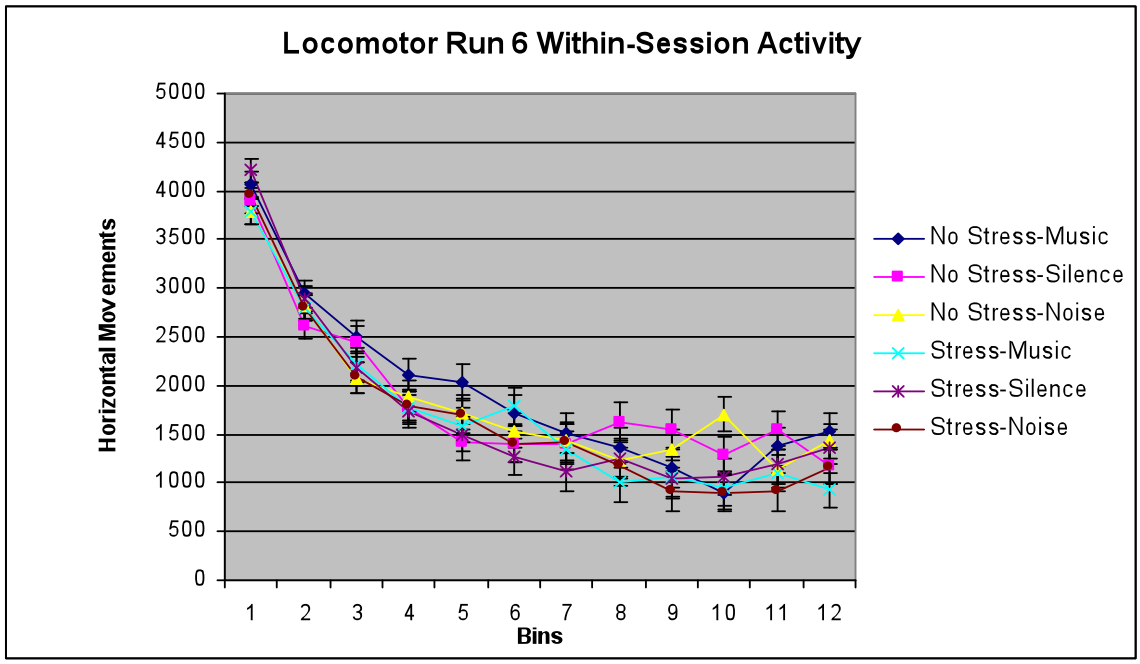

Figure 20. Locomotor Run 6 Within-Session Activity

A split-file was performed by tumor status. In animals without tumors activity decreased over time (F [6.994, 300.758] = 117.923, p < 0.001). In animals with tumors, there was also the same effect (F [6.162, 203.343] = 84.45, p < 0.001). There also was a stress effect where the stressed condition had lower activity than did the non-stressed condition (F [1, 33] = 7.272, p = 0.01). In summary, all animals habituated to the locomotor arena during the session. In animals with tumors, animals exposed to stress had a steeper decline in activity than the animals that were not exposed to stress.

Run 7 Within-Session Activity (seventh week of stress and sound manipulations). At Run 7, all conditions decreased activity over time (F [8.215, 673.653] = 244.792, p < 0.001) (see Figure 21).

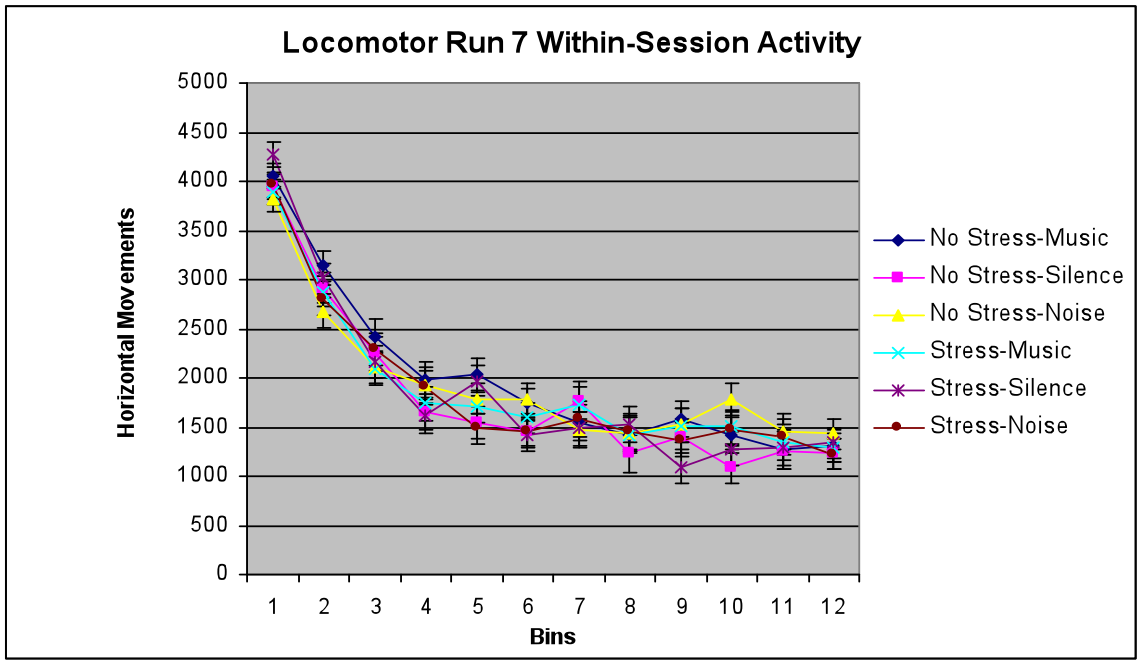

Figure 21. Locomotor Run 7 Within-Session Activity

A split-file was performed based on tumor status. In animals with tumors, activity decreased over time (F [7.209, 237.884] = 89.418, p < 0.001). There was a stress by sound interaction where the non-stressed music condition had lower activity than did the stressed music condition, but the stressed noise condition had lower activity than did the non-stressed noise condition (F [2, 33] = 3.445, p < 0.05). In animals without tumors, activity decreased over time (F [7.575, 325.705] = 125.458, p < 0.001). There was a stress by sound interaction where the stressed music condition had lower activity than did the non-stressed music condition (F [2, 43] = 4.003, p < 0.05).

In summary, all animals habituated to the locomotor arena during the session. In animals with tumors, the non-stressed music condition learned faster than did the stressed music condition, but the stressed noise condition learned faster than the non-stressed noise condition. In animals without tumors, the stressed music condition learned faster than did the non-stressed music condition.

Run 8 Within-Session Activity (eighth week of stress and sound manipulations). At Run 8, activity declined over time in all conditions ($F [8.215, 665.440] = 239.291$, $p < 0.001$) (see Figure 22).

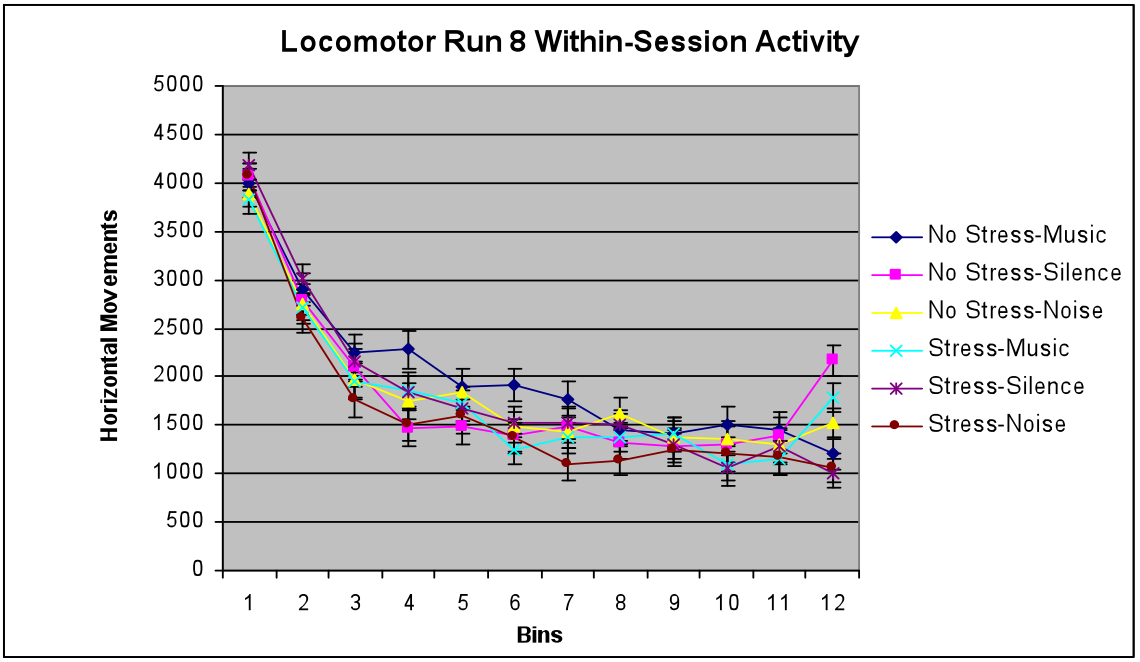

Figure 22. Locomotor Run 8 Within-Session Activity

A split-file was performed based on tumor status. In animals with tumors, activity declined over time ($F [7.085, 226.717] = 87.710$, $p < 0.001$). In animals without tumors, activity decreased over time ($F [7.209, 309.983] = 119.618$, $p <$

0.001). In animals without tumors, there was a stress by sound interaction where the stressed music condition had lower activity than did the non-stressed music condition ($F [2, 43] = 4.529$, $p < 0.05$).

In summary, all animals habituated to the locomotor arena during the session. In animals without tumors, the stressed music condition learned faster than did the non-stressed music condition.

Anxiety-Like Behavior (Center Time Ratio)

In order to be conservative when analyzing center time, so that center time was not solely based on increased horizontal activity, center time ratios were computed (center time/total activity time) and used in the following analyses. At baseline measure, there were significant differences between conditions. There was a significant main effect for stress, where the stressed animals spent more time in the center of the locomotor arena than did the non-stressed animals ($F [1, 83] = 6.511$, $p < 0.05$). Because of this initial difference, the baseline center time ratio measure was used as a covariate in subsequent analyses. Over all center time measures (see Figure 23), there was a significant sound effect where the music condition spent less time in the center than did the silence and noise conditions ($F [2, 80] = 5.786$, $p < 0.01$).

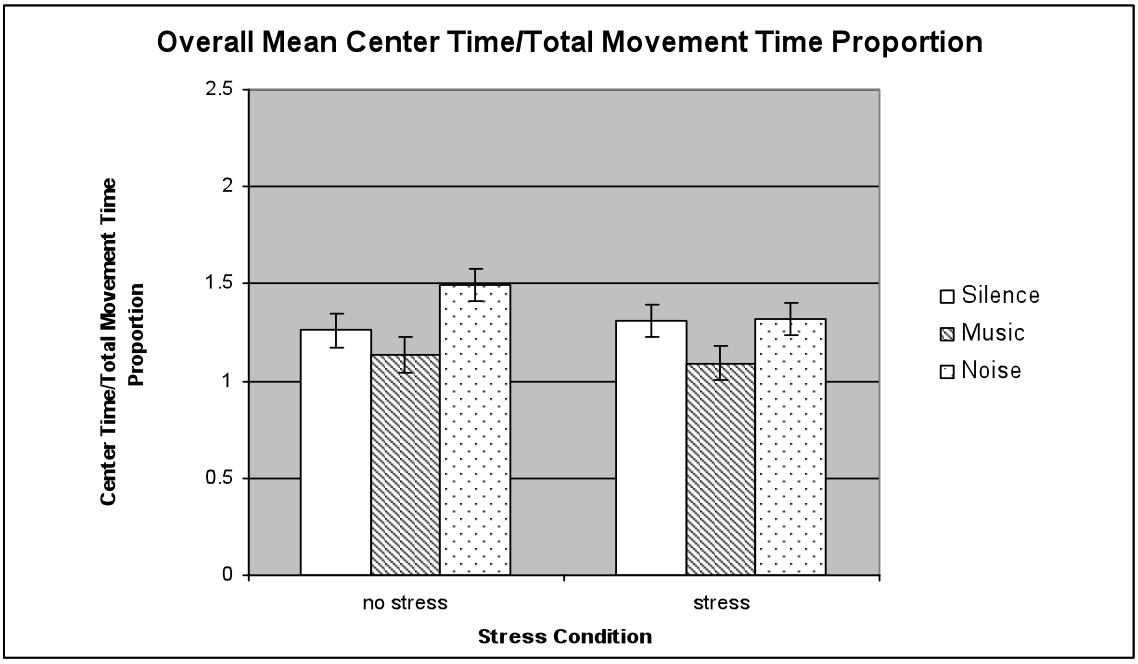

Figure 23. Overall Mean Center Time/Total Movement Time Proportion

A split-file was performed based on tumor status. There was a significant main effect for sound only in the animals with tumors (see Figure 24), where the music condition spent less time in the center than did the noise condition ($F [2, 31] = 3.377$, $p < 0.05$).

The last center time ratio was measured to determine if there were differences between conditions at the end of the experiment. While there were no significant differences overall, a split-file based on tumor status revealed a significant main effect for sound where the noise condition spent more time in the center than did the silence and music conditions only in animals with tumors.

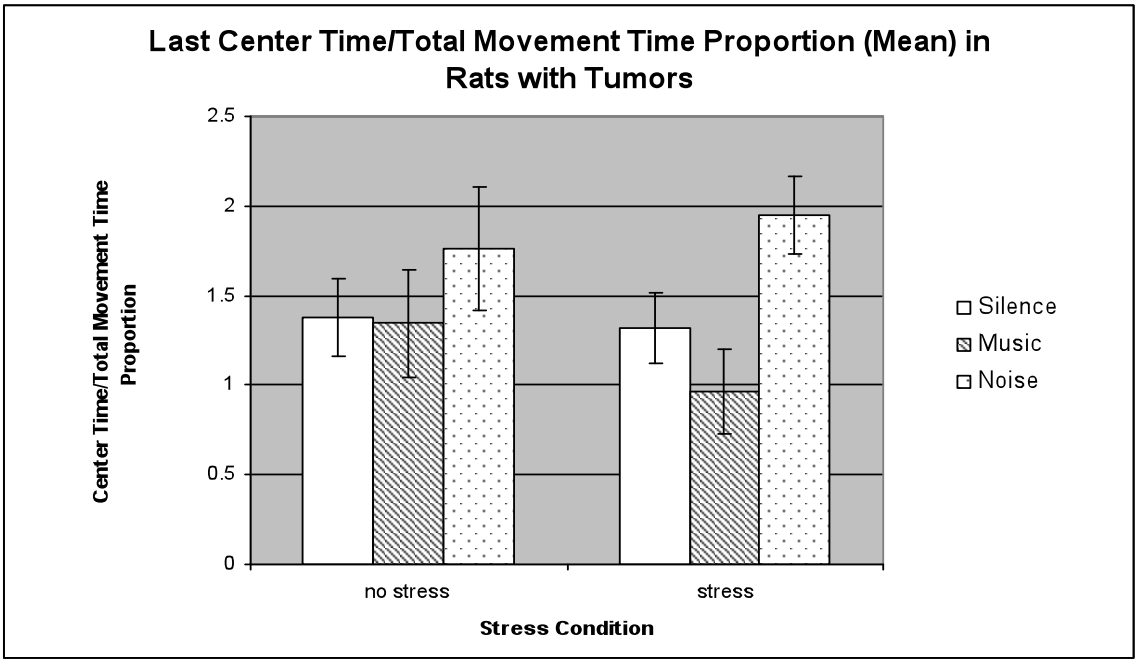

Figure 24. Last Center Time/Total Movement Time (Mean) in Rats with Tumors

In summary, there was a significant sound effect for center time. The music condition spent significantly less time in the center of the locomotor arena than the silence and noise conditions, suggesting that the music condition was more anxious. In animals with tumors, the noise condition spent the most time in the center, especially during the last measure, suggesting that the noise condition was less anxiety-producing.

Depression-Like Behavior

Horizontal Activity. There was a significant main effect for time where horizontal activity increased over the experiment in all conditions ($F [6.217, 503.589] = 36.087$, $p < 0.001$) (see Figure 25). There was a significant time by sound interaction where the noise condition increased at a faster rate than did the silence and music conditions ($F [12.434, 503.589] = 2.163$, $p < 0.05$). There

also was a significant time by stress by sound interaction but no clear pattern appeared (F [12.434, 503.589] = 2.063, p < 0.05).

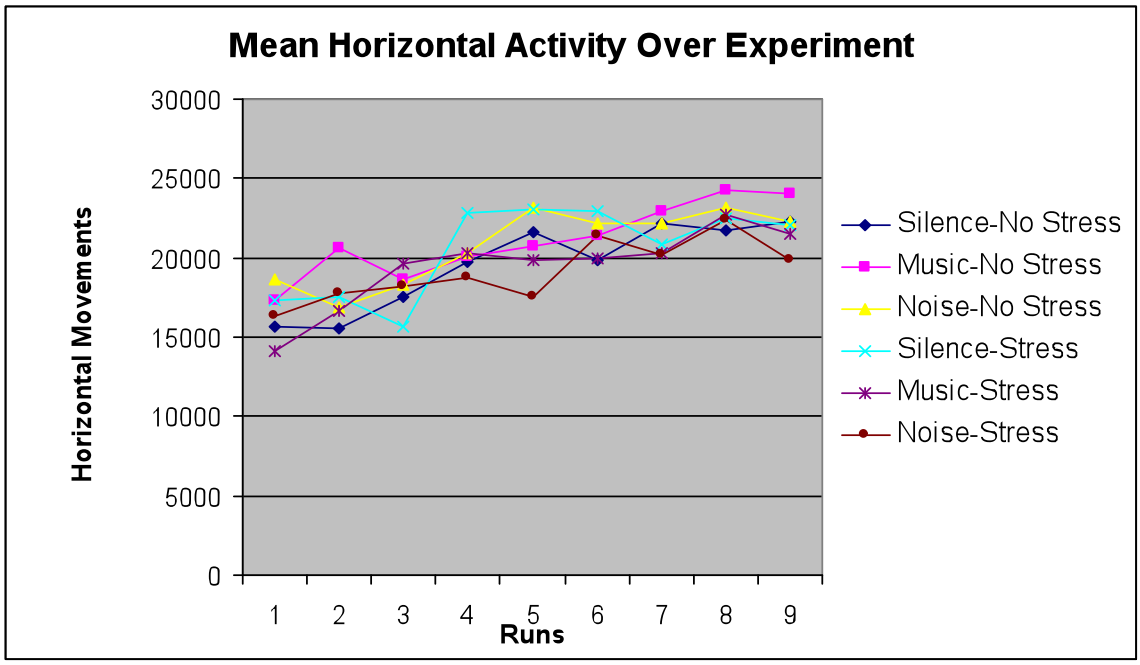

Figure 25. Mean Horizontal Activity Over Experiment

A split-file by tumor status was performed. In animals without tumors there was only a significant main effect for time where horizontal activity increased over the course of the experiment in all conditions (F [5.921, 254.614] = 20.469, p < 0.001). In animals with tumors, there also was a significant main effect for time where horizontal activity increased over time in all conditions (F [5.696, 182.285] = 15.65, p< 0.001). In animals with tumors there was a significant time by sound interaction where the noise condition increased horizontal activity at a faster rater than did the silence and music conditions (F [11.393, 182.285] = 2.33, p = 0.01). There was a significant stress by sound interaction in animals with tumors (see Figure 26) where the non-stressed music condition had lower horizontal activity than did the stressed music condition, and

the non-stress noise condition had more horizontal activity than did the stressed noise condition ($F [2, 32] = 4.108$, $p < 0.05$). There also was a significant time by stress by sound interaction in animals with tumors, however, no clear pattern emerged ($F [11.393, 182.285] = 2.12$, $p < 0.01$).

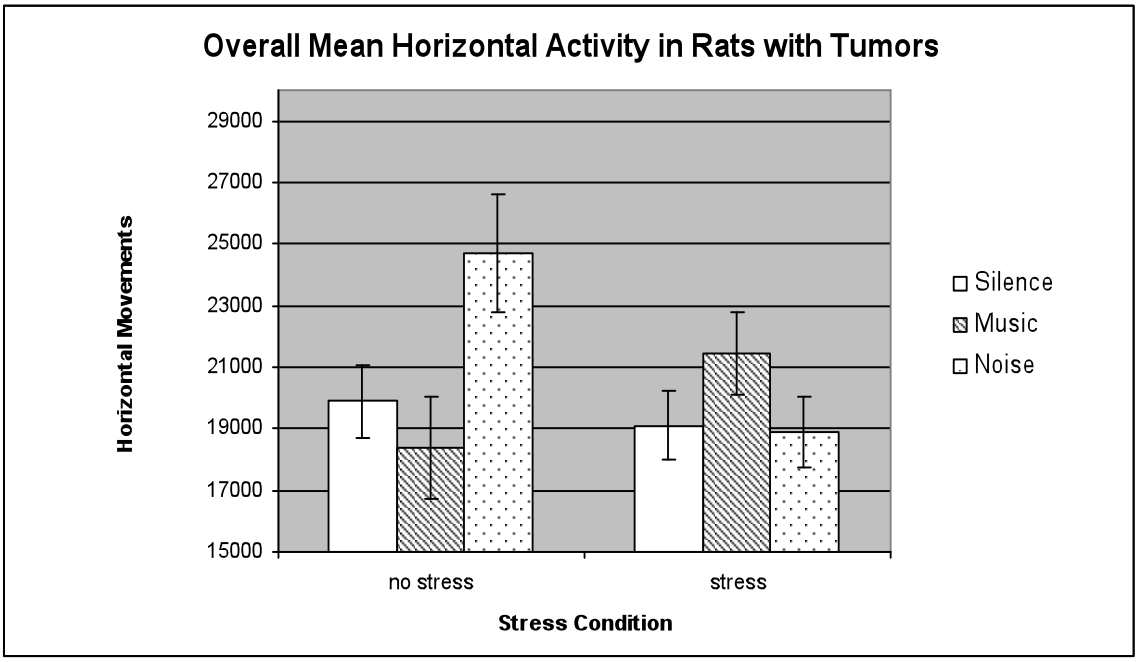

Figure 26. Overall Mean Horizontal Activity in Rats with Tumors

The last horizontal activity was analyzed to determine if there differences at the end of the experiment. There were no significant differences found between conditions. However, when a split-file was performed, there was a significant stress by sound interaction in animals without tumors (see Figure 27). In the music condition, there was greater horizontal activity when not exposed to stress than when exposed to stress ($F [2, 43] = 4.529$, $p < 0.05$).

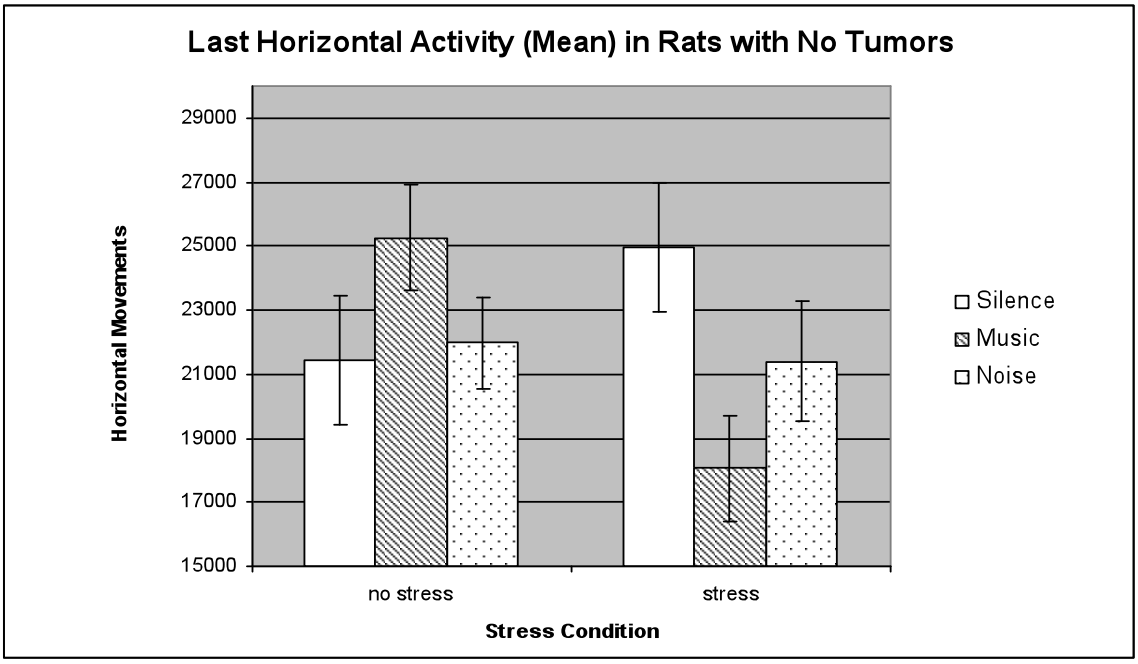

Figure 27. Last Horizontal Activity (Mean) in Rats with No Tumors

In summary, horizontal activity appeared to increase over the course of the experiment in all animals, indicating that animals did not become depressed over time. It appears that animals in the noise condition increased activity at a faster rate than animals in the silence and music conditions suggesting an increase in interest and exploration; however, this may be due solely to the effects found in animals with tumors. In addition, in animals with tumors, stress appeared to increase horizontal activity (interest) in the music condition but decrease horizontal activity in the noise condition. It appears that stress increases depression-like behavior in the noise condition in animals with tumors. At the end of the experiment, it appeared that stress increased depression-like behavior in the music condition in animals that did not have tumors.

Vertical Activity. At baseline measure, there was a significant main effect for sound where the music condition had less vertical activity than the noise condition ($F [2, 83] = 3.224$, $p < 0.05$). Because of this initial baseline difference, the baseline measure was used as a covariate in subsequent analyses.

Over the vertical activity measures, there was a significant main effect for time (see Figure 28) where vertical activity increased over time in all conditions ($F [5.276, 422.058] = 7.163$, $p < 0.001$).

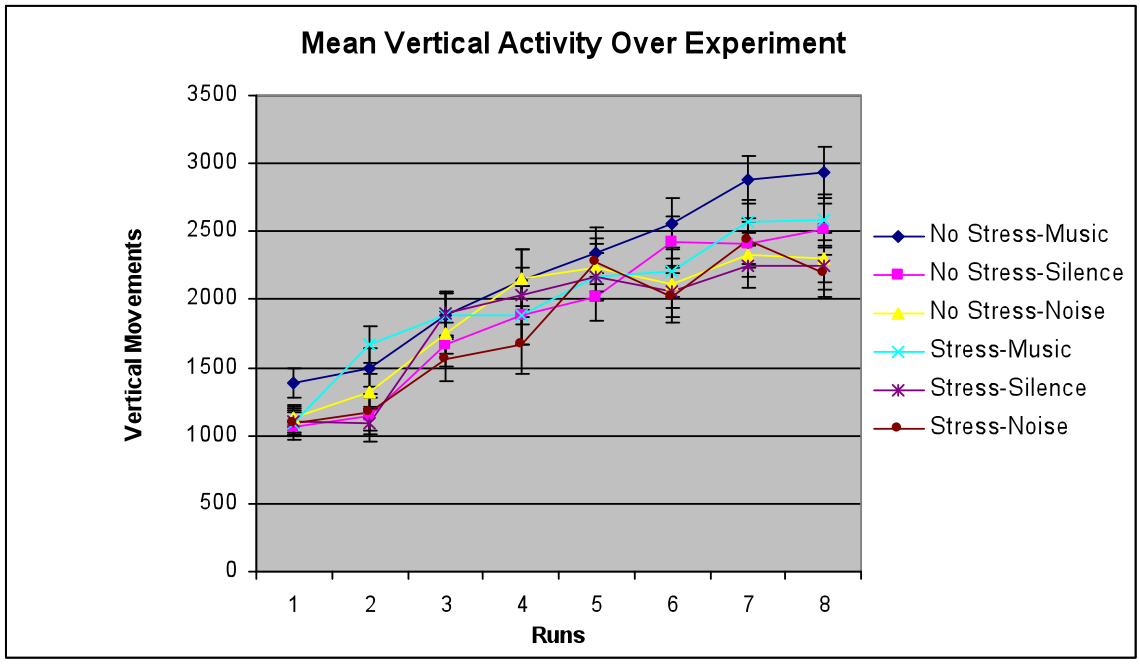

Figure 28. Mean Vertical Activity Over Experiment

A split-file was performed based on tumor status. In animals without tumors there was only a significant main effect for time where vertical activity increased over time for all conditions ($F [4,857, 204.006] = 4.111$, $p < 0.01$). Animals with tumors also had a significant main effect for time where vertical activity increased over time in all conditions ($F [4.670, 144.767] = 2.785$, $p <$

0.05). In animals with tumors there also was a significant stress by sound interaction (see Figure 29) where the non-stressed noise condition had more vertical activity than did the stressed noise condition (F [2, 31] = 3.767, p < 0.05).

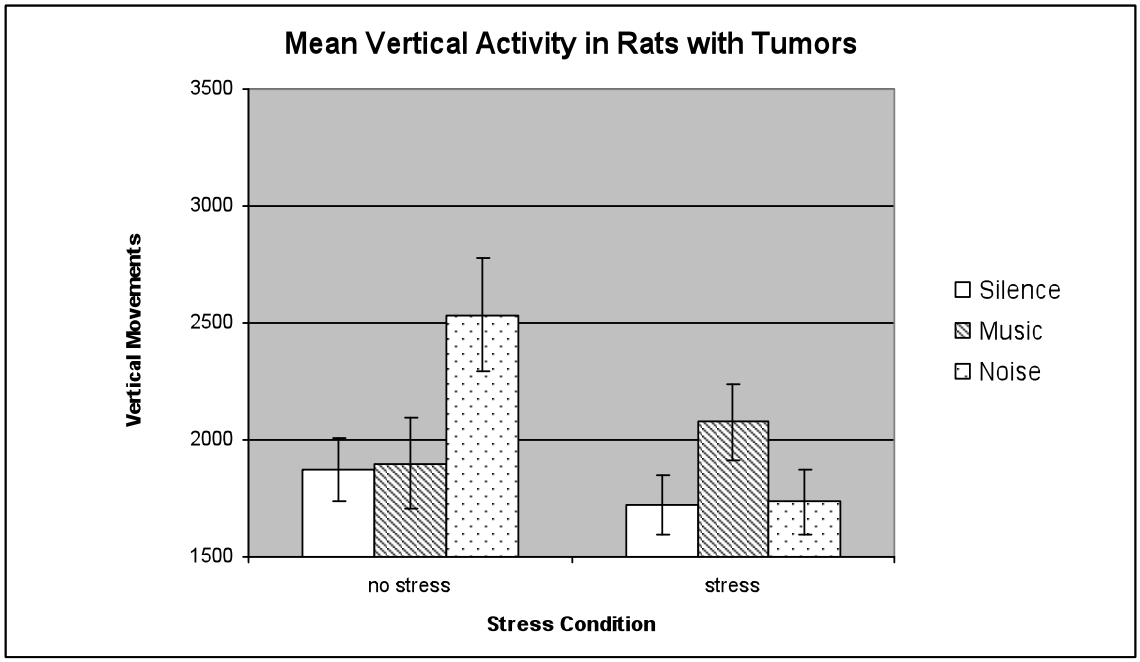

Figure 29. Mean Vertical Activity in Rats with Tumors

The last vertical activity measure was analyzed to determine if there were differences between conditions at the end of the experiment. There was a significant main effect for sound (see Figure 30) where the music condition had more vertical activity than did the silence and noise conditions (F [2, 80] = 3.810, p < 0.05).

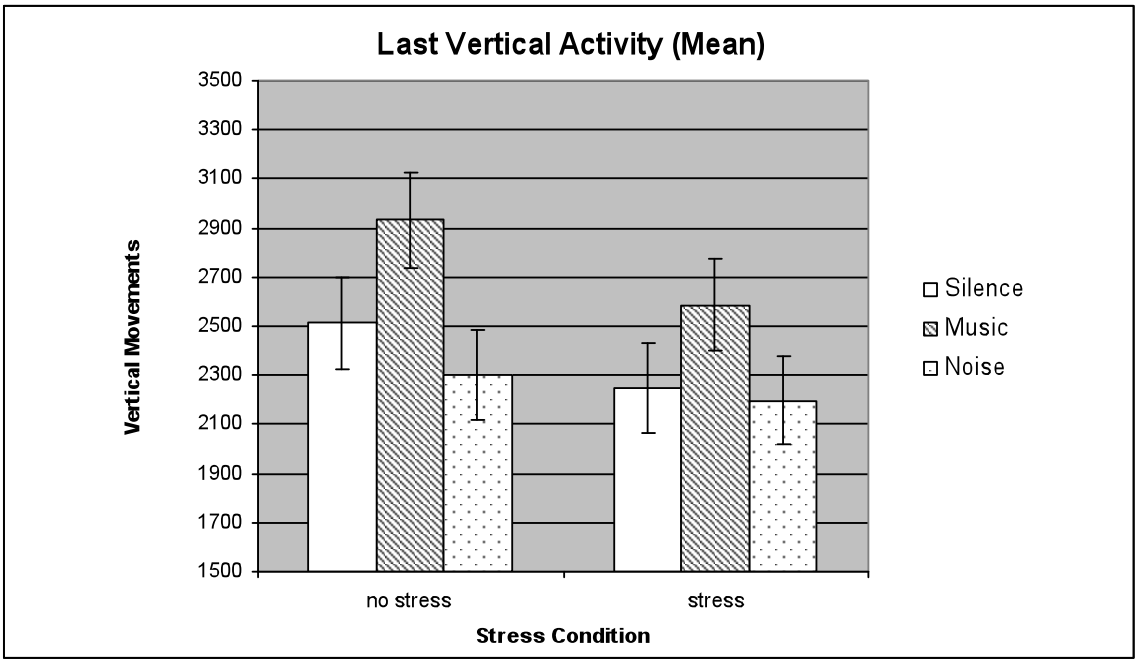

Figure 30. Last Vertical Activity (Mean)

A split-file was performed based on tumor status; however, there were no significant differences between conditions found in both animals with tumors and animals without tumors.

In summary, vertical activity increased over time in all conditions. In animals with tumors, stress decreased vertical activity in animals in the noise condition (increased depression-like behavior). At the end of the experiment, the animals in the music condition had more vertical activity than the silence and noise conditions, indicating that music may increase interest and exploration.

Ultrasonic Vocalizations (USV) Low (Negative Affect). The baseline USV Low measure had significant differences between conditions. There was a significant main effect for sound where the music condition had more USV Low than the did the silence and noise conditions ($F [2, 83] = 3.430$, $p < 0.05$). The

sound effect is better explained by the significant stress by sound interaction where the music condition had more USV Low than did the silence and noise conditions only in the non-stressed animals ($F [2, 83] = 3.194$, $p < 0.05$). Because of the initial differences at baseline, the baseline measure was used as a covariate in subsequent USV Low analyses.

Over all the USV Low measures (see Figure 31), there was a significant main effect for stress where stressed animals had lower USV Low than did non-stressed animals ($F [1, 80] = 4.780$, $p < 0.05$). There was a significant main effect for sound where the silence condition had more USV Low than did the music and noise conditions ($F [2, 80] = 5.716$, $p < 0.01$). There also was a significant time by stress interaction ($F [1.254, 100.317] = 6.962$, $p < 0.01$), time by sound interaction ($F [2.508, 100.317] = 7.289$, $p < 0.001$), and time by stress by sound interaction ($F [2.508, 100.317] = 8.281$, $p < 0.001$), but no clear patterns emerged.

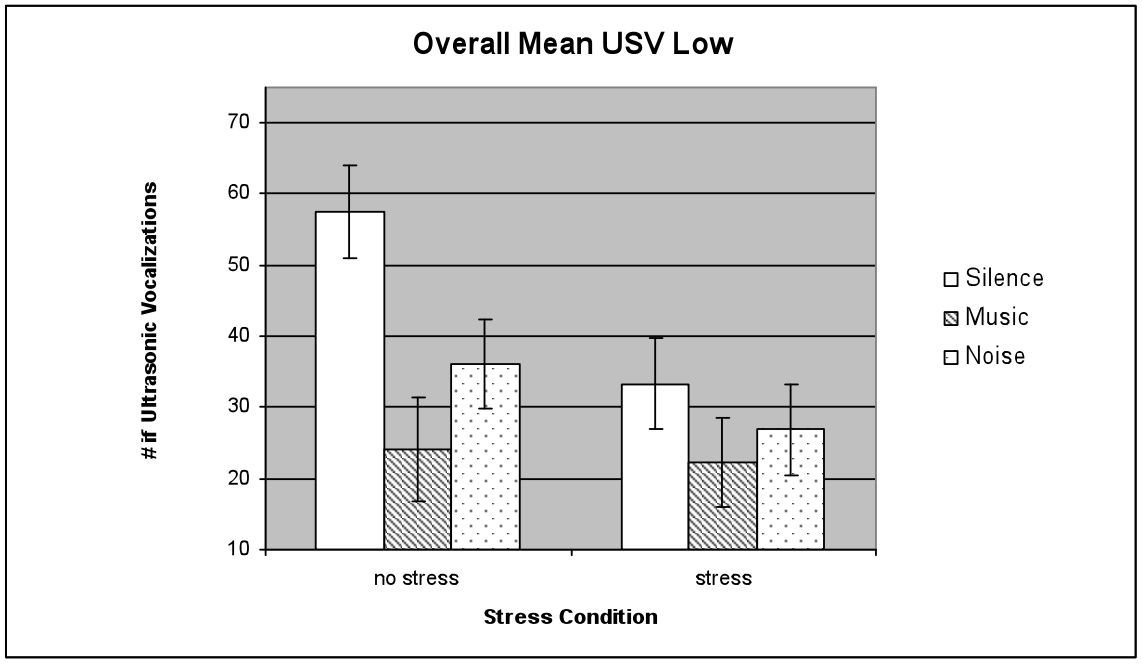

Figure 31. Overall Mean USV Low

A split-file was performed based on tumor status. There were no significant differences between conditions in animals with tumors. In animals without tumors (see Figure 32), there was a significant main effect for sound where the music condition had lower USV Low than did the noise condition, and the music and noise conditions had lower USV Low than did the silence condition ($F [2, 42] = 6.434$, $p < 0.01$). There also was a significant time by stress interaction ($F [1.535, 64.468] = 5.883$, $p < 0.01$), time by sound interaction ($F [3.070, 64.468] = 4.760$, $p < 0.01$), and time by stress by sound interaction ($F [3.070, 64.468] = 6.442$, $p < 0.001$), but no clear patterns emerged.

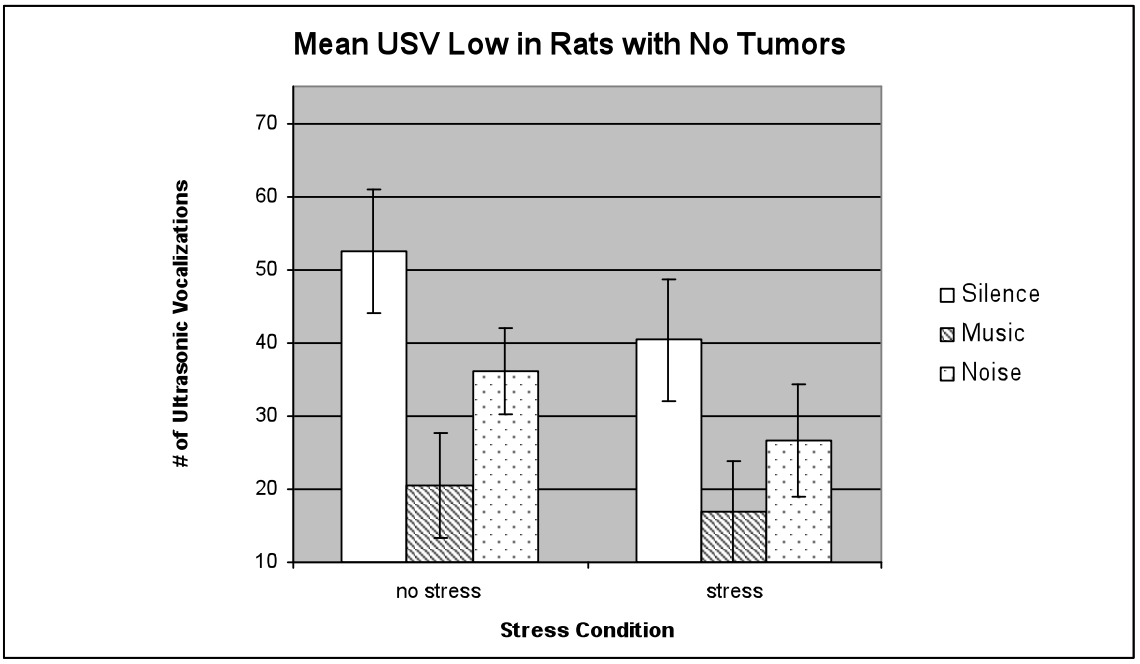

Figure 32. Mean USV Low in Rats with No Tumors

An analysis on the last USV Low measure was conducted to determine if there were any differences at the end of the experiment. There were no significant differences between conditions on the last measure of USV Low. When a split-file was performed based on tumor status, there was a significant stress by sound interaction in animals without tumors (see Figure 33) where the non-stressed noise condition had more USV Low than did the non-stressed silence and music conditions, and the stressed silence condition had more USV Low than did the stressed music and noise conditions ($F [2, 42] = 5.651$, $p < 0.01$).

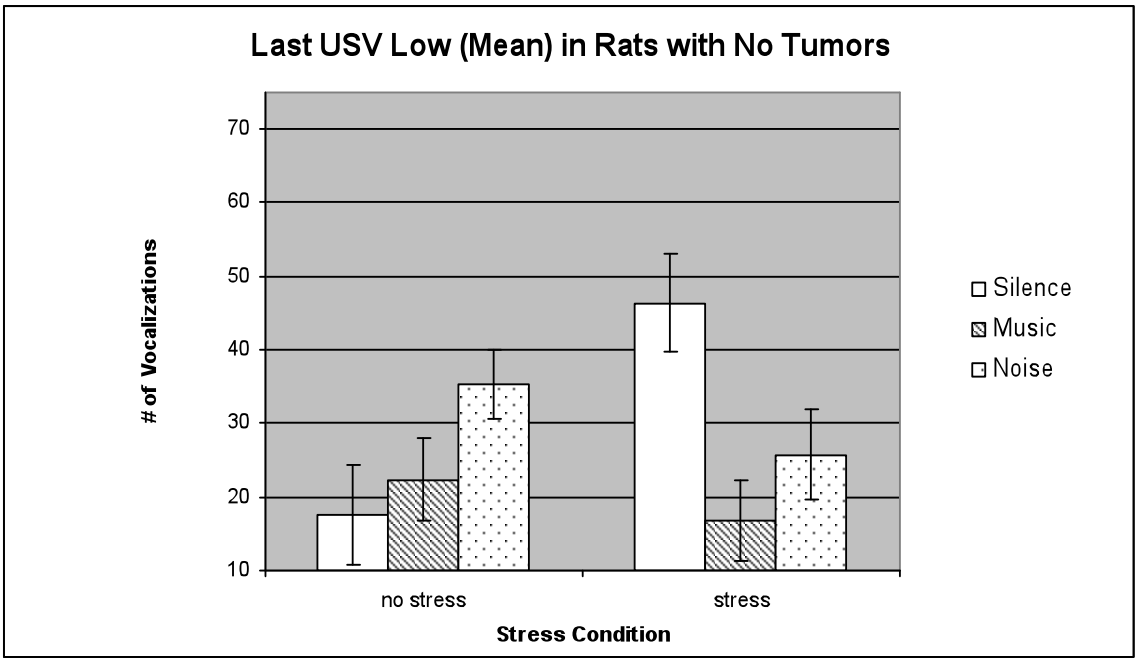

Figure 33. Last USV Low (Mean) in Rats with No Tumors

In summary, the stressed animals had lower USV Low than non-stressed animals, suggesting that non-stressed animals had more negative affect. The silence condition had more USV Low (negative affect) than did the music and noise conditions. This finding was apparent in animals without tumors. At the end of the experiment, there were only differences in animals without tumors. For non-stressed animals, the noise condition had more USV Low (negative affect) than the silence and music conditions, but for the stressed animals, the silence condition had more USV Low (negative affect) than did the music and noise conditions.

Ultrasonic Vocalizations (USV) High (Positive Affect). There were no initial differences between conditions at the baseline USV High measure. Over the USV High measures (see Figure 34), there was a significant main effect for time

where all conditions had an increase in USV High over time (F [5.229, 423.548] = 34.501, p < 0.001). There was a significant time by stress interaction (F [5.229, 423.548] = 2.773, p < 0.05), time by sound interaction (F [10.458, 423.548] = 3.915, p < 0.001), and time by stress by sound interaction (F [10.458, 423.548] = 3.433, p < 0.001), but there were no clear patterns that emerged.

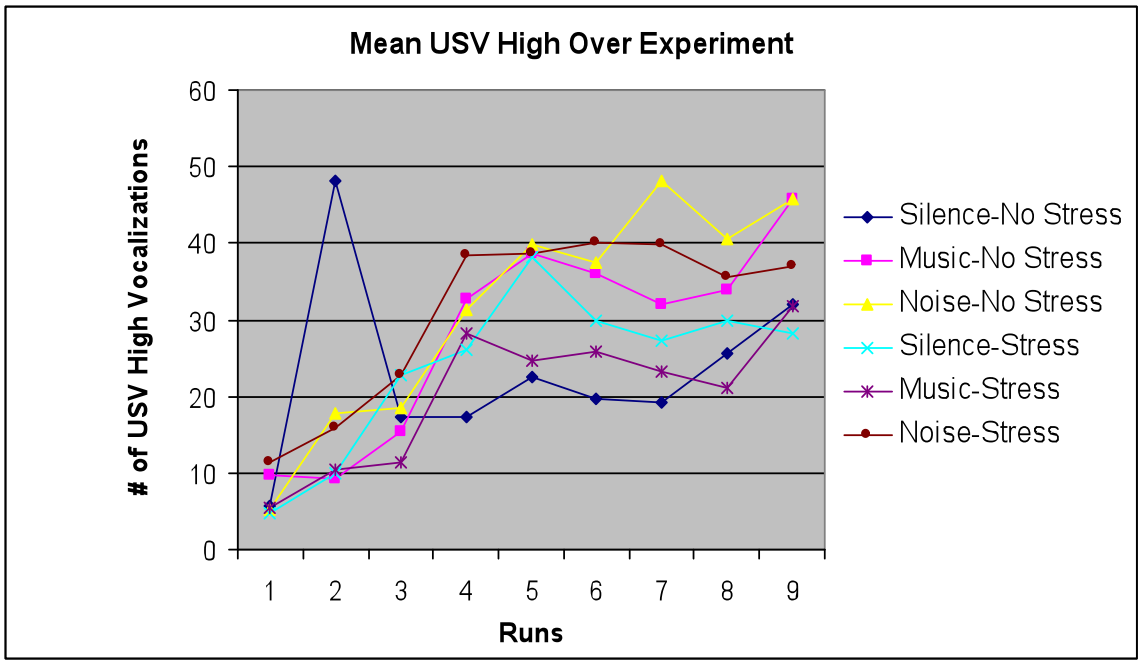

Figure 34. Mean USV High Over Experiment

A split-file was performed by tumor status. In animals with tumors, there was a significant main effect for time where USV High increased over time in all conditions (F [4.032, 129.039] = 12.356, p < 0.001). There was a significant time by sound interaction where the music and noise conditions increased USV High at a faster rate than did the silence condition (F [8.065, 129.039] = 2.523, p < 0.05). In animals without tumors, there was a significant main effect for time where USV High increased over time in all conditions (F [5.310, 228.329] =

19.950, $p < 0.001$). There was a significant time by stress interaction (F [5.310, 228.329] = 2.291, $p < 0.05$) and time by stress by sound interaction (F [10.620, 228.329] = 2.843, $p < 0.01$), but no clear pattern emerged.

The last measure of USV High was analyzed to determine if there were any significant differences between conditions at the end of the experiment. There were no significant differences between conditions. A split-file was performed based on tumor status. Again, there were no significant differences found between condition in both animals with tumors and animals without tumors.

In summary, all conditions had an increase in USV High (positive affect) over the course of the experiment. In animals with tumors, the music and noise conditions appeared to increase USV High (positive affect) at a faster rate than did the silence condition. There were no differences between conditions, regardless of tumor status, at the last measure of USV High.

Hypotheses Revisited

Hypothesis 1a: **Stress will increase body weight and food consumption.**

Stress did not increase body weight. There were no differences in body weight between stress conditions. In fact, in animals with tumors, stressed animals had lower body weights than non-stressed animals. Stress did not increase food consumption. There were no differences in food consumption between stress conditions. Hypothesis 1a was **not confirmed**.

Hypothesis 1b: **Exposure to music will attenuate body weight gain and food consumption.**

Exposure to music did not attenuate body weight gain. There were differences in body weight between sound conditions. Music did attenuate food consumption compared to the noise and silence conditions. This effect was seen in animals without tumors. Hypothesis 1b was **partially confirmed**.

Hypothesis 1c: **Exposure to music will attenuate the effects of stress on body weight and food consumption compared to noise exposure and no music/noise control groups.**

Overall, exposure to music did not attenuate the effects of stress on body weight because there were no effects on body weight. However, in rats with tumors, it appears that music attenuated body weight gain in rats in the stressed condition compared to animals in the stressed silence condition. Music did not attenuate the effects of stress on food consumption. In fact, animals without tumors in the music condition consumed more food than did animals in the noise and silence conditions. Hypothesis 1c was **partially confirmed**.

Table C. Hypothesis 1 Results

VARIABLE	OVERALL	WITH TUMORS	WITHOUT TUMORS
Body Weight	↑ over time	↑ over time	↑ over time
		no stress > stress	
		No stress: S<M&N	
		Stress: S>M&N	
Food Consumption	↑ over time	--	M<N
	S&N ↑ faster than M		No stress: S>M&N
	M<S&N		Stress: S<M&N

S = silence; M = music; N = noise

Hypothesis 2a: **Stress will increase serum corticosterone in rats. Stress will increase adrenal gland weights and decrease spleen weights.**

Overall, stress did not increase serum corticosterone in rats. However, stress did increase serum corticosterone in animals in the silence and music conditions. Stress did not increase adrenal gland weights as there were no differences between conditions, and stress did not decrease spleen weights as there were no differences between conditions. Hypothesis 2a was **partially confirmed**.

Hypothesis 2b: **Exposure to music will lower corticosterone, attenuate adrenal gland weights, and increase spleen weight compared to exposure to noise or no music/noise.**

Exposure to music did not lower serum corticosterone. Exposure to noise lowered serum corticosterone compared to the music and silence conditions. Exposure to music did not attenuate adrenal gland weights and did not increase spleen weights. Hypothesis 2b was **not confirmed**.

Hypothesis 2c: **Exposure to music will attenuate stress' effects on corticosterone levels and spleen and adrenal gland weights.**

Exposure to music did not attenuate stress' effects on corticosterone levels, spleen weights, and adrenal gland weights. Hypothesis 2c was **not confirmed**.

Table D. Hypothesis 2 Results

VARIABLE	OVERALL	WITH TUMORS	WITHOUT TUMORS
Serum Corticosterone	N<S&M	--	N<S&M
	Stress: N<S&M		Stress: N<S&M
	S&M: no stress < stress		S&M: no stress < stress
Spleen Weights	--	--	--
Adrenal Glands Weights	--	--	--

S = Silence; M = music; N = noise

Hypothesis 3a: **Stress will decrease the duration until detection of first tumor occurrence, increase the number of animals that develop tumors (incidence), increase the number of tumors present (multiplicity), hasten tumor growth, and will have larger tumors/tumor spread (end tumor weight).**

Stress did not decrease the duration until detection of first tumor occurrence because there were no differences between groups. Stress did not increase the number of animals that developed tumors; however, there was a trend where there were more animals that developed tumors in the stressed noise condition than in the non-stressed noise condition. Overall, stress did not increase the number of tumors present, although stress noticeably increased tumor multiplicity in the noise condition. Stress did not increase tumor growth (although in the noise group, stress hastened growth compared to the non-stressed group), or end tumor weight. Hypothesis 3a was **not confirmed**.

Hypothesis 3b: **Exposure to music will attenuate time until first tumor, will decrease tumor incidence, will decrease the number of tumors, will attenuate tumor growth, and will decrease tumor weight.**

Exposure to music did not attenuate time until first tumor, did not decrease tumor incidence, did not decrease the number of tumors, did not attenuate tumor growth, and did not decrease tumor weight. There was a trend where animals in the music condition had a smaller percentage of tumor presence than animals in the silence condition; however, this difference was not statistically significant. Hypothesis 3b was **not confirmed**.

Hypothesis 3c: **Exposure to music will attenuate the effects of stress on time until first tumor detection, tumor incidence, tumor multiplicity, tumor growth, and tumor weight.**

Exposure to music did not attenuate the effects of stress on time until first tumor detection, tumor incidence, tumor multiplicity, tumor growth, and tumor weight. Hypothesis 3c was **not confirmed**.

Table E. Hypothesis 3 Results

VARIABLE	**FINDINGS**
Tumor Incidence	Noise: stress > no stress **
Tumor Multiplicity	No stress-Noise ↓ **
Time to First Tumor Detection	--
Tumor Growth	No stress: N<M&S **
Tumor Weight	--

S = silence; M = music; N = noise; ** = trend

Hypothesis 4a: **Stress will decrease horizontal activity and interfere with within-session activity habituation.**

Stress did not decrease horizontal activity. Stress did not interfere with within-session activity habituation. In fact, in a couple of locomotor runs, in animals with tumors, animals that were stressed had a steeper habituation curve than did the non-stressed animals. Hypothesis 4a was **not confirmed**.

Hypothesis 4b: **Exposure to music will increase horizontal activity and enhance within-session habituation.**

Exposure to music did not increase horizontal activity and overall did not enhance within-session habituation. Only during the baseline locomotor measure, music enhanced within-session habituation compared to the silence and noise conditions in animals with tumors only. Hypothesis 4b **partially confirmed**.

Hypothesis 4c: **Exposure to music will attenuate the effects of stress on horizontal activity and within-session activity habituation.**

Overall, exposure to music did not attenuate the effects of stress on horizontal activity. However, in the animals with tumors, music exposure increased horizontal activity in the stressed condition. While there was no clear pattern, exposure to music in stressed animals steepened the habituation curve in Run 2 in the animals with tumors, and in Runs 7 and 8 in animals without tumors. Hypothesis 4c was **partially confirmed**.

Table F. Hypothesis 4 Results

VARIABLE	OVERALL	WITH TUMORS	WITHOUT TUMORS
Horizontal Activity	↑ over time	↑ over time	↑ over time
	N ↑ faster over time than S&M	N ↑ faster over time than S&M	
Baseline Within-Session	↓ over time	↓ over time	↓ over time
		M<S<N	
Run 1 Within-Session	↓ over time	↓ over time	↓ over time
	M&N<S		Music: stress < no stress
	Music: stress < no stress		Silence: no stress < stress
Run 2 Within-Session	↓ over time	↓ over time	↓ over time
		stress < no stress	
		S<M<N	
		Noise: stress < no stress	
Run 3 Within-Session	↓ over time	↓ over time	↓ over time
		No stress: N>S&M	
		Stress: N<S&M	
		Music: no stress < stress	
		Noise: stress < no stress	
Run 4 Within-Session	↓ over time	↓ over time	↓ over time
	stress < no stress	Noise: stress < no stress	
Run 5 Within-Session	↓ over time	↓ over time	↓ over time
Run 6 Within-Session	↓ over time	↓ over time	↓ over time
		stress < no stress	
Run 7 Within-Session	↓ over time	↓ over time	↓ over time
		No Stress: M&S<N	
		Stress: S&N<M	Music: stress < no stress
		Music: no stress < stress	No stress: S&N<M
		Noise: stress < no stress	Stress: M<S&N
Run 8 Within-Session	↓ over time	↓ over time	↓ over time
			Music: stress < no stress

S = silence; M = music; N = noise

Hypothesis 5a: **Stress will increase anxiety-like behaviors (decreased center time).**

Stress did not increase anxiety-like behaviors. Hypothesis 5a was **not confirmed**.

Hypothesis 5b: **Exposure to music will decrease anxiety.**

Exposure to music did not decrease anxiety. In fact, exposure to music had less center time activity than the silence and noise conditions. During the last measure, the noise condition had more center time activity in animals with tumors. Hypothesis 5b was **not confirmed**.

Hypothesis 5c: **Exposure to music will attenuate the effects of stress on anxiety.**

Exposure to music did not attenuate the effects of stress on anxiety. Hypothesis 5c was **not confirmed**.

Table G. Hypothesis 5 Results

VARIABLE	OVERALL	WITH TUMORS	WITHOUT TUMORS
Center Time	M<N&S	M<N	--

S = silence; M = music; N= noise

Hypothesis 6a: **Stress will increase depressive-like behaviors (increase ultrasonic vocalizations low – negative affect, decrease ultrasonic vocalizations high – positive affect, and decrease horizontal and vertical activity).**

Stress did not increase negative affect. Stress did not decrease positive affect. Overall, stress did not decrease horizontal activity, although stress did

decrease horizontal activity in the noise condition in animals with tumors. Overall, stress did not decrease vertical activity, although it decreased vertical activity in the noise condition in animals with tumors. Hypothesis 6a was **partially confirmed**.

Hypothesis 6b: **Exposure to music will decrease depressive-like behaviors compared to noise or no music/noise.**

Exposure to music did decrease negative affect compared to the silence condition. This effect was especially apparent in animals without tumors. Exposure to music and noise increased positive affect compared to the silence condition in animals with tumors. Exposure to music did not increase horizontal activity. Exposure to noise led to a faster increase in horizontal activity compared to the silence and music conditions. At the end of the experiment, exposure to music increased vertical activity compared to the silence and noise conditions. Hypothesis 6b was **partially confirmed**.

Hypothesis 6c: **Exposure to music will attenuate the effects of stress on depressive behaviors.**

Overall, exposure to music did not attenuate the effects of stress on negative affect; however, at the end of the experiment rats exposed to music and noise had lower amounts of negative affect than those in the silence condition. Exposure to music did not attenuate the effects of stress on positive affect. Exposure to music appeared to attenuate the effects of stress on horizontal activity in animals with tumors because they had increased horizontal activity. But at the end of the experiment, music did not attenuate the effects of stress on

horizontal activity in animals without tumors because they had decreased horizontal activity. Exposure to music did not attenuate the effects of stress on vertical activity. Hypothesis 6c was **partially confirmed**.

Table H. Hypothesis 6 Results

VARIABLE	OVERALL	WITH TUMORS	WITHOUT TUMORS
USV Low	stress < no stress	--	M<N<S
	M&N < S		
USV High	↑ over time	↑ over time	↑ over time
		N&M ↑ faster than S	Music: no stress > stress over time
Horizontal Activity	↑ over time	↑ over time	↑ over time
	N ↑ faster over time than S&M	N ↑ faster over time than S&M	
Vertical Activity	↑ over time	↑ over time	↑ over time
		Noise: stress < no stress	

S = silence; M = music; N = noise

Table I. Hypotheses Findings

HYPOTHESIS	FINDING
1A	Not Confirmed
1B	Partially Confirmed
1C	Partially Confirmed
2A	Partially Confirmed
2B	Not Confirmed
2C	Not Confirmed
3A	Not Confirmed
3B	Not Confirmed
3C	Not Confirmed
4A	Not Confirmed
4B	Partially Confirmed
4C	Partially Confirmed
5A	Not Confirmed
5B	Not Confirmed
5C	Not Confirmed
6A	Partially Confirmed
6B	Partially Confirmed
6C	Partially Confirmed

In summary, there were some findings that partially supported the hypotheses. With regard to body weight, music attenuated body weight gain in animals without tumors. Music also attenuated body weight gain in the stressed condition. With regard to serum corticosterone, stress increased serum corticosterone in the silence and music conditions. For within-session activity, music enhanced within-session habituation in animals with tumors at the baseline measure. Additionally, music enhanced within-session habituation in the stressed condition during Run 2 in animals with tumors and during Runs 7 and 8 in animals without tumors suggesting that music may have been slightly beneficial at improving simple learning during these runs. For depression-like measures, stress decreased horizontal activity and vertical activity in the noise condition in animals with tumors (increased depression). Music increased positive affect (USV High) in animals with tumors, music increased vertical activity during the last measure (increased interest and exploration), and music increased horizontal activity (increased interest) in animals with tumors in the stressed condition.

In addition, there also were some findings that were not hypothesized. With regard to body weight, the non-stressed noise condition increased body weight gain in animals with tumors. With regard to food consumption, noise increased food consumption in stressed animals with no tumors. Serum corticosterone levels were lower in the non-stressed noise condition than in the stressed noise condition. The non-stressed noise condition had a trend of lower tumor incidence, lower tumor multiplicity, and slower tumor growth. The noise

condition increased center time (decreased anxiety) in animals with tumors. The noise condition increased horizontal activity (increased exploration and interest) in animals with tumors. The noise condition decreased negative affect (decreased USV Low) in animals without tumors and increased positive affect (increased USV High) in animals with tumors.

Discussion

The present experiment examined the effects of stress and sound conditions on indices of distress (e.g., anxiety and depressive symptoms) and tumor progression. This experiment is the first to examine these variables in a MNU-induced mammary cancer model in rats. There are several interesting findings.

Body Weight

All animals gained weight over the course of the experiment. However, animals with tumors gained less weight in the silence condition compared to the music and noise conditions when not stressed. In contrast, when stressed, the animals gained more weight in the silence condition than in the music and noise conditions. It is clear that the **sound conditions influenced body weight gain in animals with tumors, where perhaps music and noise are beneficial when not stressed but are detrimental when experiencing stress**. These changes in body weight gain in animals with tumors were not the result of changes in food consumption.

Food Consumption

All animals had a gradual increase in food consumption over the time of the experiment. However, the music condition increased food consumption at a slower rate than in the silence and noise conditions. In animals without tumors, when not exposed to stress the silence condition consumed more food than the other sound conditions but when exposed to stress they consumed less food than the other sound conditions. Sound condition influenced food consuming behavior. **It appears as if, at least in animals without tumors, the sound conditions may have been stressful in animals not exposed to stress, but the sound may have buffered the effects of stress on food consumption for animals exposed to stress**.

Serum Corticosterone

As expected, **there was a stress effect in serum corticosterone where there are higher levels in the stressed condition and lower levels in the unstressed condition in the silence and music conditions**. However, **the reverse occurred in the noise condition** where serum corticosterone levels were higher in the unstressed condition and lower in the stressed condition. Noise by itself may have been a stressor, yet combined with stress it may have acted as a buffer. These effects appear to occur in animals without tumors, because there were no significant differences in serum corticosterone in animals with tumors. It may be that animals with tumors were experiencing biological changes from the cancer and that is why the various conditions did not impact levels of serum corticosterone.

Tissue Measures

There were no significant differences between conditions for spleen weight. There were no significant differences between conditions for adrenal glands weight. While there were no statistically significant differences between conditions for tumor incidence, there was a notable trend. **The silence condition had the greatest percentage of tumor incidence compared to the other sound conditions**. Interestingly, **the non-stressed, noise condition had the lowest percentage of tumor incidence** especially when compared to the stressed, noise condition. It appears that **when not exposed to stress, noise may be beneficial in terms of tumor occurrence**. There were no significant differences between conditions in end tumor weight. There were no significant differences between conditions when first tumor detection occurred. While there were no significant differences between conditions in tumor growth rate, there was a trend where tumor growth was slower in animals in the noise condition that were not exposed to stress, a similar pattern that was seen in tumor incidence. Again, while not statistically significant, **noise may be beneficial in animals not exposed to stress in terms of tumor growth**. There were no significant differences between conditions in tumor multiplicity, and again, an interesting pattern appeared where **the lowest tumor multiplicity was the noise condition that was not exposed to stress**.

Within-session Activity

Within-session locomotor activity had no clear, evident pattern in terms of effects of the various conditions. While within session locomotor activity is an

index of simple learning, it did not support the Mozart effect literature. However, most of the Mozart effect literature specifically addresses effects on spatial-learning and, therefore, within-session activity may not have been a good indicator to try to replicate improvement in learning.

Anxiety

In terms of center time, an index for anxiety, the music condition spent significantly less time in the center of the locomotor arena than the silence and noise conditions. This result suggests that music does not decrease a behavioral indicator of anxiety in rats. In animals with tumors, those in the noise condition seem to spend the most time in the center, especially during the last measure. **It may be that noise decreases anxiety in rats with tumors**.

Depression

Horizontal activity appeared to increase over the course of the experiment in all animals, indicating that animals did not become depressed over time, regardless of their condition. It appears that animals in the noise condition increased activity at a faster rate than did animals in the silence and music conditions suggesting an increase in interest and exploration; however, this effect may be due solely to the effects found in animals with tumors. This effect suggests that **noise may be beneficial for animals with cancer in terms of a behavioral index of depression**. However, when looking at animals that were stressed versus not stressed, it appears that stress increases depression-like behavior in the noise condition in animals and music decreases depression-like behavior in animals with tumors. However, based on the last measure, it

appears that stress increased depression-like behavior in the music condition in animals that did not have tumors. It is clear that sound condition and stress condition alter horizontal activity dependent on whether an animal has cancer or not.

Vertical activity increased over time in all conditions. In animals with tumors, stress decreased vertical activity in animals in the noise condition (increased depression-like behavior). At the end of the experiment, the animals in the music condition had more vertical activity than the silence and noise conditions, indicating that **music may increase interest and exploration**.

Contrary to expectation, stressed animals had lower USV Low (an indicator of negative affect) than non-stressed animals. The silence condition had more USV Low than the music and noise conditions. This finding was apparent in animals without tumors. This finding suggests that **music and noise may be beneficial in decreasing negative affect in animals without tumors**. At the end of the experiment, animals not exposed to stress in the noise condition had more USV Low than the silence and music conditions, but for the stressed animals, the silence condition had more USV Low than the music and noise conditions. This finding suggests that **when animals are not exposed to stress, noise may be stressful increasing negative affect, but when exposed to stress music and noise may be beneficial**. Perhaps, the exposure to sounds buffered the effects of stress.

All conditions had an increase in USV High over the course of the experiment, suggesting an increase in positive affect. In animals with tumors, the

music and noise conditions appeared to increase USV High at a faster rate than the silence condition. **It may be that music and noise increase positive affect particularly in animals with tumors**.

These findings present a similar picture to what is often found among humans. Specifically, it is not uncommon to have biological and behavioral patterns that are not consistent with each other. For example, a person may report an improvement in anxiety but may continue to show high levels of biological stress indicators. Additionally, these findings support differences in conditions based on cancer status. Cancer, in and of itself, is a physical stressor that has a large impact on the immune system and biological processes. Therefore, different responses based on tumor status, makes sense. It is also difficult to know whether there were differences in response that made an animal vulnerable to develop tumor versus not, or if the differences were due to the cancer.

Major findings in animals that developed tumors include: sound increased weight gain; serum corticosterone did not differ depending on condition; noise decreased anxiety symptoms; noise may have been helpful with regard to tumor incidence, tumor growth, and tumor multiplicity in non-stressed animals; stress may increase tumor incidence; and when stressed, sound has inconsistent results on depression measures. The major finding in animals without tumors was that noise attenuated serum corticosterone when not stressed.

Limitations

As is true with all experiments, there are limitations to this experiment. An animal experiment may not be an accurate portrayal of the human condition. The method of cancer induction is not necessarily a perfect substitute for how women develop breast cancer. In fact, the cancer induction method did not produce the desired number of animals with cancer (e.g., it was expected that all animals would develop tumors, however, this effect did not occur).

One animal had developed tumors by the fourth week. Six more animals developed tumors by the sixth week. Eight more animals developed tumors by the seventh week. Sixteen animals developed tumors by the eighth week. Eight weeks of stress/sound was the initial plan for experimental duration. However, only 31 out of 89 animals had developed tumors by the end of the originally planned experimental period. Therefore, the experiment was extended for two weeks to determine if extending the duration of the study would produce more animals that developed tumors. By the ninth week, three more animals had developed tumors, but by the tenth week only one more animal developed tumors. The experiment was ended after ten weeks (two weeks longer than originally planned and five weeks longer than tumors should have appeared according to Dr. Thompson). On the day of euthanasia it was discovered that five more animals had developed tumors, but the tumors were too small to detect via external palpation.

Because approximately half of the animals (i.e., 40 out of 89) developed tumors, the power of the study was not adequate for many analyses.

Additionally, based of the variability of animals that had tumors in each condition, animals that developed tumors could not be compared statistically with animals that did not develop tumors. Rather, the analyses had to rely on performing a split-file function which does not allow for this direct comparison. In addition, the recurrent stressors used in the proposed experiment are not a perfect substitute for the stressors that impact women with breast cancer, as they may experience stress more frequently, less frequently, etc. Perhaps, the method of stress induction was not severe enough to adequately portray the constant stress that a person with breast cancer would experience. It may have been more effective to use a stressor that was more severe. In addition, the particular model of mammary cancer has similarities to human breast cancer, but this model also has differences. The MNU model of mammary cancer is in a rat of comparable age to young women and may not be applicable to breast cancer in women who are older.

The music manipulation was based on the few pieces of literature that were available. Because a spatial-learning task was not included, it is difficult to know if the music manipulation replicated past findings in the animal literature. However, Dr. Rauscher was consulted about music selection and she (an expert in the Mozart Effect in animals and humans) agreed with the musical selections that were used. Also, because the rats were triple housed, based on past studies using the MNU model of mammary cancer, this housing may have altered the effects of stress as social housing may attenuate effects of stress

(e.g., social support), and it may have influenced other aspects such as activity level (Brown & Grunberg, 1995).

Because animals were triple-housed, the food consumption measure is not exact to an individual animal; rather it is based on the amount of food consumed by all animals in a given cage. It would be impossible to determine the amount of food an individual animal consumes when freely fed if they are socially housed. Although the measure is not specific to a given animal, food consumption is analyzed in a way that this method decreases variance among the measures (since the average food consumption per condition, not animal, is analyzed). If there were large differences in food consumption per animal in a cage then body weight measures would allow this concern to be identified. There were no marked discrepancies between food consumption and body weight.

Based on these limitations future studies should consider these issues. It may be more appropriate to begin experimental manipulation after all animals have developed tumors to determine how the stress and sound manipulations affect indices of distress and tumor progression in animals with defined tumors. Future studies may include a maze learning task to determine if the music manipulation replicates findings from previous studies. Also, different music selections should be tried in future studies. Additionally, it would be valuable to try different stress paradigms to determine if there are differences based on the magnitude of stress because the current stressors may have been too mild. It also might be useful to include individual food consumption measures.

Another point worth noting was that there were several significant differences among treatment groups during baseline measures. The number of significant findings at baseline (when no differences were expected) raises concern because of the improbability of these differences occurring by chance. However, baseline differences in behavior are quite common in small sample-sized animal experiments. It is important to note that precautions were taken to minimize the impact of baseline differences on the results. Animals in this experiment were randomly assigned to conditions on the first day of the study to try to prevent the chance of spurious findings. Additionally, baseline measures of body weight were not significantly different, which was important because MNU injections to induce mammary tumors are based on body weight. To statistically control for baseline differences that did occur, baseline measures were used as covariates whenever significant baseline differences occurred. While the overall main findings in this experiment are thought to be actual significant findings rather than spurious findings, replication is important to confirm these results. Future studies can match subjects on several dependent variables, rather than rely on the "purist" model of random assignment.

Clinical Implications

Based on the present experiment's findings there may be several clinical implications. For people with breast cancer experiencing recurrent, acute stressors, listening to music or noise (e.g., white noise machine) may help with body weight gain. Additionally, listening to music may increase interest and exploration (i.e., decrease depressive symptoms); however, listening to noise

may decrease interest and exploration. For people with breast cancer that may not be experiencing recurrent, acute stressors, listening to music or noise may increase positive affect. Listening to noise may protect against tumor incidence, may slow tumor growth, and may slow tumor multiplicity. It is relevant to note that the "noise" used in this study consisted of periods of white noise of varying intensity similar to the music and was intended to serve as a sound control. This "noise" was similar to the background sound of a "sound shield" and perhaps, sounds of a wave machine or other nature sounds. In fact, the "noise" sounded the wind and similar sounds of nature. Noise, or more accurately, sounds that have a rhythmic quality but lack certain musical qualities, may be beneficial for relaxation. In fact, noise in the form of nature sounds or background sounds are currently sold for the purposes of relaxation and may indeed have some merit behind their use.

For people without breast cancer but who have been exposed to a cancer-causing risk (e.g., carcinogen), listening to noise might decrease biochemical measures of stress (e.g., corticosterone) if they are experiencing recurrent, acute stressors. Additionally, listening to music and noise might decrease negative affect, and listening to music might increase interest in activities. However, listening to noise could potentially decrease interest in activities. For people with risk (e.g., exposure to carcinogens) but who do not experience recurrent, acute stressors, music might decrease negative affect. Listening to noise might decrease tumor incidence, however it could also increase biochemical measures of stress such as corticosterone.

At this point, there is no clear cut advice on what intervention an individual should use. The interventions should be tailored to an individual based on cancer status and whether they are experiencing daily stressors. Additionally, a specific intervention could be positive in one aspect but negative in another. It would be important to weigh the pros and cons for each individual. It does seem, however, that sound other than music *per se* should be further examined for effects on stress and tumors.

Conclusions

There are several interesting findings worth noting. The most unusual finding was that less than half of the animals in this experiment developed tumors. This finding was surprising given the fact that the literature and discussions with Dr. Thompson suggest that most, if not all animals, develop tumors in response to the MNU injection. For the present experiment, the procedures were directly learned from Dr. Thompson and the directions for MNU injection were followed as instructed. However, there are several possible reasons for the discrepancy.

It is likely that the husbandry and care of animals were different in the present experiment compared with research in Dr. Thompson's laboratory. In the present experiment, the investigator handled all husbandry matters (i.e., cage changes, cage cleaning, water changing, and food changing). The husbandry procedures in the Thompson laboratory were not observed, and therefore, are not known. In terms of housing environment, the Thompson laboratory did not operate with a reverse light cycle. The present experiment operated with a 12-

hour reverse-light cycle because of the use of behavioral measures and the desire to not interfere with the animals' sleep cycle (rats are nocturnal animals). Most of the Thompson studies focused on the use of dietary interventions, rather than behavioral measures. It is possible that the differences in diet, as well as the addition of behavioral measures, alter mammary carcinoma development and progression. Additionally, the present experiment had a stress manipulation. While there have been studies of exercise, and perhaps exercise-induced stress, in the Thompson laboratory, the difference in stress manipulation also may have played a role in tumor development and progression (Thompson, 1994). The combination of gentling plus behavioral measures in the present experiment meant that the animals were handled and taken out of their housing room more often than in other studies using the MNU model. The increased handling and exposure to different environments may have altered the development of tumors in animals. Interestingly, an experiment using a chemically-induced cancer model in Sprague-Dawley rats that also included substantial animal handling and behavioral measures similarly resulted in about half the tumor incidence expected based on published studies that did not include handling and behavioral measures (Patricia Deuster, personal communication, February 25, 2011). Perhaps, future studies can determine if handling of animals or engaging in a variety of behaviors significantly alters tumor development.

While the present experiment had the expectations of tumor development in all animals, there are benefits to the fact that approximately half of the animals developed tumors. First, the absence and presence of tumors could clearly be

detected in the animals. This finding is important because it suggests that the MNU model of mammary cancer can be used to detect tumor development and progression. Also, this finding allowed the experimenter to examine differences in animals that had tumors and differences in animals that did not have tumors with regard to the effects of the stress and sound manipulations.

One of the most notable results of this experiment was the effect of the noise manipulation. Initially, the noise condition was included as a sound control. The noise was purposely manipulated to mimic the varying intensities of the music condition. Both the noise and the Mozart selections had a rhythmic quality, but the noise lacked melodic properties that were present in the Mozart selections. Despite the absence of these musical properties, the noise condition altered behavioral and biological measures. In fact, many of the noise effects suggest that noise (or nature sounds) may have positive effects on animals. Most music research done has not included a sound control condition. Based on the present experiment, future studies should examine the effects of various sounds and noises on behavioral and biological measures.

This experiment was the first to utilize behavioral measures and sound manipulations in the MNU-model of mammary cancer with rats. The results of this experiment suggest that the use of the MNU-model with behavioral measures and behavioral interventions relevant to breast cancer is valuable. This experiment also used a sophisticated sound control (the “noise” condition) that is generally lacking in the research that examines the use of music interventions. Though the literature in the use of sound interventions for breast

cancer is relatively sparse, several experts (Dr. Henry Thompson, expert in the MNU-model, and Dr. Frances Rauscher, expert in the Mozart Effect) were contacted to improve the design and execution of this experiment. The MNU-model of mammary cancer was learned first-hand by watching and performing the preparation, administration, and disposal of the chemical carcinogen in the Thompson laboratory at Colorado State University. Dr. Frances Rauscher was consulted to aid in the selection of Mozart compositions for this experiment. By consulting with the experts in these areas, this experiment was designed and performed with precision to contribute meaningfully to the existing literature.

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APPENDIX A – IACUC Memo

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4712
http://www.usuhs.mil

January 20, 2010

MEMORANDUM FOR DR.NEIL GRUNBERG, DEPARTMENT OF MEDICAL AND CLINICAL PSYCHOLOGY

SUBJECT: IACUC Approval of Protocol – Initial Review

The following application was reviewed and approved by the Uniformed Services University of the Health Sciences (USUHS) Institutional Animal Care and Use Committee (IACUC) via Full Committee Review on January 20, 2010:

Title of Application: "Biobehavioral Effects of Stress on Mammary Cancer in Rats"

USUHS Protocol Number: MPS-10-743

Expiration Date: January 19, 2013

Supporting Grant(s) Number: R072JK

Name of Principal Investigator: Dr. Neil Grunberg

The USUHS has an Animal Welfare Assurance on file with the Office for Laboratory Animal Welfare (OLAW), National Institutes of Health (NIH). The Assurance Number is A3448-01. The IACUC approved the above referenced application as submitted.

An annual review is required for each of the three years of this protocol. This review must be completed by the anniversary date of the protocol. If work is to be continued past the expiration date, a triennial review must be completed prior to the expiration date in order for work to be uninterrupted. Protocol expiration dates may not be extended, and no animal work may be done without an approved protocol. Although the IACUC may send reminders, it is the investigator's responsibility to submit an annual review form (Form 3206A) at least 30 days in advance, or a new Form 3206 for triennial review at least 60 days in advance of expiration.

Prior to placing your first animal order, please contact Ms. Megan Ralls to schedule a pre-protocol planning meeting (295-3579). This meeting must occur to ensure animal numbers are loaded in the CART system and LAM resources are available to meet your needs.

Brian M. Cox, Ph.D.
Chair, Institutional Animal
Care and Use Committee

cc:
Office of Research

Learning to Care for Those in Harm's Way

APPENDIX B – Laboratory Pictures

Picture 1. Locomotor Chamber

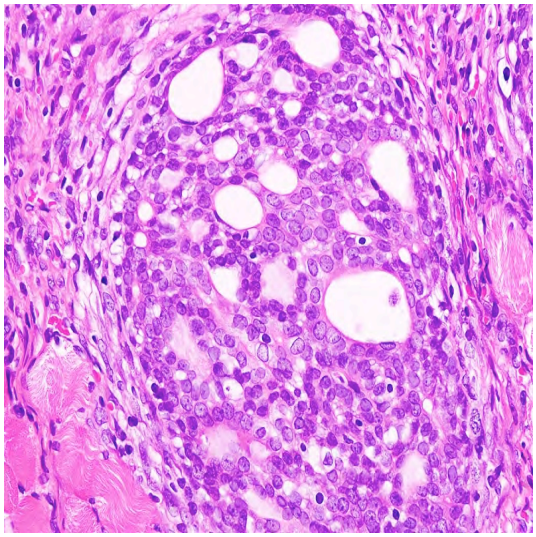
Picture 2. USV

Picture 3. MNU Preparation

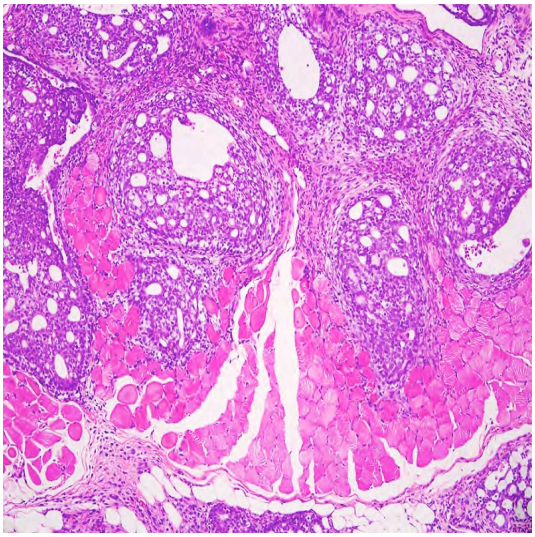
Picture 4. Fume Hood

Picture 5. Rats at 21 Days Old

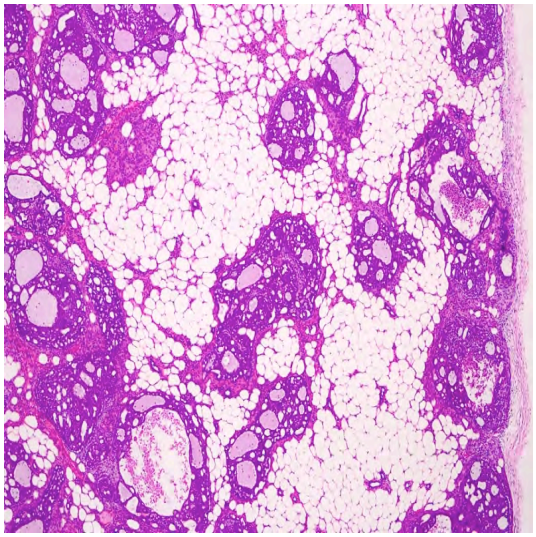
APPENDIX C – Sample of Histopathology Pictures from Tumors in Experiment



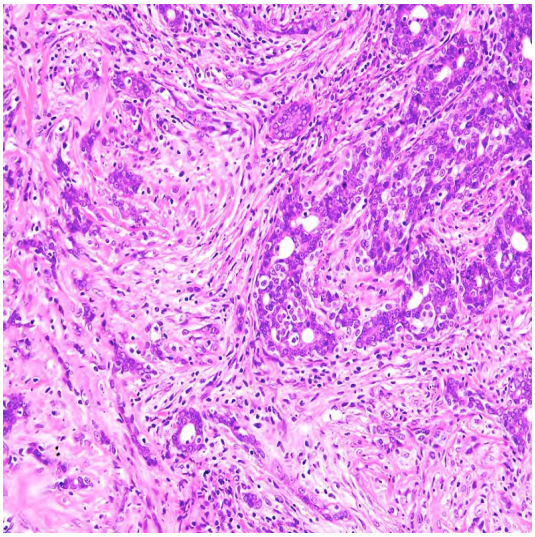
Mammary adenocarcinoma-Cribriform pattern (glands)-400X



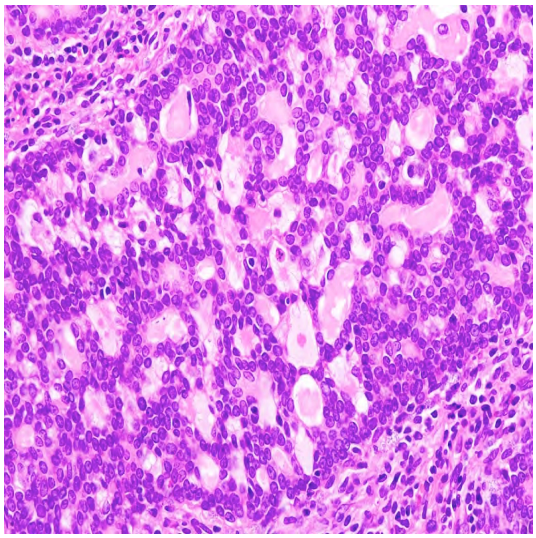
Mammary adenocarcinoma -invading muscle(Pink) -100X



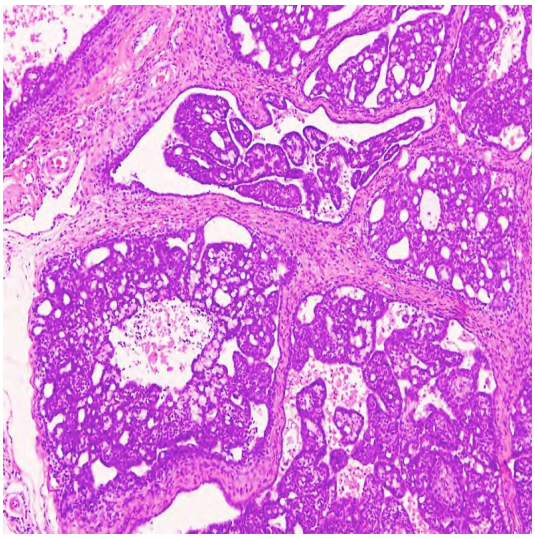
Ductal origin-of-mammary adenocarcinoma-40X



Scirrhous adenocarcinoma-area-200X



Acinar adenocarcinoma mammary-400X
-gland-like structures



Papillary adenocarcinoma
(left and upper left)-along side Cribriform areas-
100X

APPENDIX D – Statistical Tables

List of Statistical Tables

Table 68. Last Vertical Activity (Split File by Tumor)

TABLES

Table 1. Body Weight (initial)	
ANOVA	
Stress	F(1, 83) = 0.464, p = 0.497
Sound	F(2, 83) = 0.791, p = 0.457
Stress x Sound	F(2, 83) = 1.938, p = 0.150

Table 2. Body Weight Overall			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.000	44	0.000	0.196

Repeated Measures ANOVA	Sphericity Violated (G.G.)
Time	F(1.767, 143.155) = 4711.696, p = 0.000
Time x Stress	F(1.767, 143.155) = 0.589, p = 0.536
Time x Sound	F(3.535, 143.155) = 0.634, p = 0.619
Time x Stress x Sound	F(3.535, 143.155) = 1.716, p = 0.157
Stress	F(1, 81) = 1.145, p = 0.288
Sound	F(2, 81) = 0.471, p = 0.626
Stress x Sound	F(2, 81) = 2.675, p = 0.075

Table 3. Body Weight (Split File by Tumors)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.000	44	0.000	0.249
No Tumors	0.000	44	0.000	0.157

Repeated Measures ANOVA	With Tumors (G.G.)	Without Tumors (G.G.)
Time	F(2.244, 71.817) = 1844.628, p = 0.000	F(1.411, 60.652) = 2643.276, p = 0.000
Time x Stress	F(2.244, 71.817) = 2.917, p =0.055	F(1.411, 60.652) = 0.605, p = 0.494

Time x Sound	F(4.489, 71.817) = 0.569, p = 0.705	F(2.821, 60.652) = 0.653, p = 0.575
Time x Stress x Sound	F(4.489, 71.817) = 3.638, p = 0.003	F(2.821, 60.652) = 0.287, p = 0.823
Stress	F(1, 32) = 4.251, p = 0.047	F(1, 43) = 0.009, p = 0.923
Sound	F(2, 32) = 0.943, p = 0.400	F(2, 43) = 0.468, p = 0.629
Stress x Sound	F(2, 32) = 6.746, p = 0.004	F(2, 43) = 0.033, p = 0.967

Table 4. Last Body Weight	
ANOVA	
Stress	F(1, 81) = 0.578, p = 0.449
Sound	F(2, 81) = 0.271, p = 0.763
Stress x Sound	F(2, 81) = 2.684, p = 0.074

Table 5. Last Body Weight (Split File by Tumors)		
ANOVA	With Tumors	Without Tumors
Stress	F(1, 32) = 4.092, p = 0.52	F(1, 43) = 0.016, p = 0.901
Sound	F(2, 32) = 0.976, p = 0.388	F(2, 43) = 0.554, p = 0.579
Stress x Sound	F(2, 32) = 6.545, p < 0.01	F(2, 43) = 0.119, p = 0.888

Table 6. Food Consumption (initial)	
ANOVA	
Stress	F(1, 83) = 12.336, p = 0.001
Sound	F(2, 83) = 6.175, p = 0.003
Stress x Sound	F(2, 83) = 5.463, p = 0.006

Table 7. Food Consumption Overall			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.001	35	0.000	0.287

Repeated Measures ANCOVA	G.G.
Time	F(2.294, 183.497) = 4.014, p = 0.015
Time x Covariate	F (2.294, 183.497) = 1.631, p = 0.194
Time x Stress	F(2.294, 183.497) = 2.415, p = 0.084
Time x Sound	F(4.587, 183.497) = 3.235, p = 0.010
Time x Stress x Sound	F(4.587, 183.497) = 2.442, p = 0.041
Covariate	F(1, 80) = 65.641, p = 0.000
Stress	F(1, 80) = 0.202, p = 0.654
Sound	F(2, 80) = 3.447, p = 0.037
Stress x Sound	F(2, 80) = 2.693, p = 0.074

Table 8. Food Consumption (Split File by Tumors)

Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.000	35	0.000	0.254
No Tumors	0.000	35	0.000	0.247

Repeated Measures ANCOVA	With Tumors (G.G.)	Without Tumors (G.G.)
Time	F(2.029, 62.902) = 1.612, p = 0.207	F(1.979, 83.137) = 2.268, p = 0.110
Time x Covariate	F(2.029, 62.902) = 0.986, p = 0.38	F(1.979, 83.137) = 2.104, p = 0.129
Time x Stress	F(2.029, 62.902) = 1.741, p = 0.183	F(1.979, 83.137) = 2.974, p = 0.057
Time x Sound	F(4.058, 62.902) = 1.533, p = 0.203	F(3.959, 83.137) = 2.115, p = 0.087
Time x Stress x Sound	F(4.058, 62.902) = 1.896, p = 0.121	F(3.959, 83.137) = 2.348, p = 0.062
Covariate	F(1, 31) = 15.063, p = 0.001	F(1, 42) = 48.811, p = 0.000
Stress	F(1, 31) = 1.135, p = 0.295	F(1, 42) = 3.237, p = 0.079
Sound	F(2, 31) = 1.152, p = 0.329	F(2, 42) = 4.052, p = 0.025 (M <N)
Stress x Sound	F(2, 31) = 0.027, p = 0.974	F(2, 42) = 8.258, p = 0.001

Table 9. Last Food Consumption	
ANCOVA	
Covariate	F (1, 80) = 19.369, p = 0.000
Stress	F(1, 80) = 0.034, p = 0.854
Sound	F(2, 80) = 0.052, p = 0.949
Stress x Sound	F(2, 80) = 3.502, p = 0.035

Table 10. Last Food Consumption (Split File by Tumors)		
ANCOVA	With Tumors	Without Tumors
Covariate	F(1, 31) = 2.329, p = 0.137	F(1, 42) = 17.225, p = 0.000
Stress	F(1, 31) = 0.719, p = 0.403	F(1, 42) = 3.278, p = 0.077
Sound	F(2, 31) = 0.084, p = 0.919	F(2, 42) = 0.579, p = 0.565
Stress x Sound	F(2, 31) = 0.913, p = 0.412	F(2, 42) = 5.535, p = 0.007

Table 11. Spleen Weight	
ANOVA	
Stress	F(1, 83) = 0.27, p = 0.869
Sound	F(2, 83) = 0.424, p = 0.656
Stress x Sound	F(2, 83) = 0.062, p = 0.94

Table 12. Spleen Weight (Split File by Tumor)		
ANOVA split file by tumor	With Tumors	Without Tumors
Stress	F(1, 34) = 0.170, p = 0.683	F(1, 43) = 0.023, p = 0.879
Sound	F(2, 34) = 0.031, p = 0.970	F(2, 43) = 0.832, p = 0.442
Stress x Sound	F(2, 34) = 0.305, p = 0.739	F(2, 43) = 0.152, p = 0.860

Table 13. Adrenal Glands Weight	
ANOVA	
Stress	F(1, 83) = 1.12, p = 0.293
Sound	F(2, 83) = 0.66, p = 0.519
Stress x Sound	F(2, 83) = 1.127, p = 0.329

Table 14. Adrenal Glands Weight (Split File by Tumor)		
ANOVA split file by tumor	With Tumors	Without Tumors
Stress	F(1, 34) = 0.769, p = 0.387	F(1, 43) = 0.044, p = 0.835
Sound	F(2, 34) = 0.421, p = 0.660	F(2, 43) = 0.120, p = 0.887
Stress x Sound	F(2, 34) = 0.67, p = 0.518	F(2, 43) = 0.215, p = 0.807

Table 15. Serum Corticosterone	
ANOVA	
Stress	F(1, 83) = 0.413, p = 0.522
Sound	F(2, 83) = 5.806, p = 0.008
Stress x Sound	F(2, 83) = 3.727, p = 0.028

Table 16. Serum Corticosterone (Split File by Tumor)		
ANOVA split file by tumor	With Tumors	Without Tumors
Stress	F(1, 34) = 0.112, p = 0.740	F(1, 43) = 0.650, p = 0.424
Sound	F(2, 34) = 1.366, p = 0.269	F(2, 43) = 7.215, p = 0.002
Stress x Sound	F(2, 34) = 0.207, p = 0.814	F(2, 43) = 6.943, p = 0.002

Table 17. Tumor Multiplicity
Kruskal-Wallis Test
Chi-Square (5) = 5.951, p = 0.311

Table 18. Time To Event (Tumor Occurrence)
Kaplan Meier Survival Analysis
Log Rank Chi-Square (5) = 5.976, p = 0.309

Table 19. Tumor Growth (All Subjects)
Kruskal-Wallis Test
Chi-Square (5) = 6.712, p = 0.243

Table 20. Tumor Growth (Subjects With Tumors Only)	
ANOVA (linear log transformation)	
Stress	F(1, 34) = 0.002, p = 0.961
Sound	F(2, 34) = 2.747, p = 0.078
Stress x Sound	F(2, 34) = 2.340, p = 0.068

Table 21. Tumor Weight	
ANOVA (linear log transformation)	
Stress	F(1, 34) = 0.000, p = 0.987
Sound	F(2, 34) = 2.277, p = 0.118
Stress x Sound	F(2, 34) = 1.987, p = 0.153

Table 22. Tumor Incidence	
Pearson's Chi-Square	
Stress	Chi-Square (1) = 1.399, p = 0.237
Sound	Chi-Square (2) = 3.319, p = 0.190
Binary Logistic Regression	
Stress x Sound	Wald Chi-Square (2) = 2.225, p = 0.329

Table 23. Horizontal Activity (baseline)	
ANOVA	
Stress	F(1, 83) = 1.643, p = 0.204
Sound	F(2, 83) = 1.526, p = 0.223
Stress x Sound	F(2, 83) = 2.361, p = 0.101

Table 24. Horizontal Activity Overall			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.311	35	0.000	0.777
Repeated Measures ANOVA		G.G.	
Time		F(6.217, 503.589) = 36.087, p = 0.000	
Time x Stress		F(6.217, 503.589) = 1.59, p = 0.145	
Time x Sound		F(12.434, 503.589) = 2.163, p = 0.011	
Time x Stress x Sound		F(12.434, 503.589) = 2.063, p = 0.017	
Stress		F(1, 81) = 0.80, p = 0.374	
Sound		F(2, 81) = 0.049, p = 0.953	
Stress x Sound		F(2, 81) = 1.007, p = 0.370	

Table 25. Horizontal Activity (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.165	35	0.030	0.712
No Tumors	0.247	35	0.014	0.740
Repeated Measures ANOVA		With Tumors (G.G.)		Without Tumors (G.G.)
Time		F(5.696, 182.285) = 15.65, p = 0.000		F(5.921, 254.614) = 20.469, p = 0.000
Time x Stress		F(5.696, 182.285) = 2.021, p = 0.069		F(5.921, 254.614) = 1.069, p = 0.381
Time x Sound		F(11.393, 182.285) = 2.33, p = 0.010		F(11.843, 254.614) = 1.412, p = 0.162
Time x Stress x Sound		F(11.393, 182.285) = 2.12, p = 0.008		F(11.843, 254.614) = 1.671, p = 0.075
Stress		F(1, 32) = 1.025, p = 0.319		F(1, 43) = 0.072, p = 0.79
Sound		F(2, 32) = 1.411, p = 0.259		F(2, 43) = 0.311, p = 0.734
Stress x Sound		F(2, 32) = 4.108, p = 0.026		F(2, 43) = 3.029, p = 0.059

Table 26. Locomotor Baseline Within-Session			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.016	65	0.000	0.542
Repeated Measures ANOVA		G.G.	
Time		F(5.965, 495.063) = 170.775, p = 0.000	
Time x Stress		F(5.965, 495.063) = 1.763, p = 0.105	
Time x Sound		F(11.929, 495.063) = 0.706, p = 0.746	
Time x Stress x Sound		F(11.929, 495.063) = 2.606, p = 0.002	
Stress		F(1, 83) = 1.643, p = 0.204	
Sound		F(2, 83) = 1.526, p = 0.223	
Stress x Sound		F(2, 83) = 2.361, p = 0.101	

Table 27. Locomotor Baseline Within-Session (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.005	65	0.000	0.501
No Tumors	0.008	65	0.000	0.509
Repeated Measures ANOVA		With Tumors (G.G.)		Without Tumors (G.G.)
Time		F(5.510, 187.340) = 75.857, p = 0.000		F(5.598, 240.734) = 75.803, p = 0.000
Time x Stress		F(5.510, 187.340) = 1.617, p = 0.151		F(5.598, 240.734) = 1.335, p = 0.245
Time x Sound		F(11.020, 187.340) = 0.687, p = 0.750		F(11.197, 240.734) = 0.762, p = 0.680
Time x Stress x Sound		F(11.020, 187.340) = 1.804, p = 0.056		F(11.197, 240.734) = 1.173, p = 0.306
Stress		F(1, 34) = 0.456, p = 0.504		F(1, 43) = 1.369, p = 0.248
Sound		F(2, 34) = 4.841, p = 0.014		F(2, 43) = 0.052, p = 0.950
Stress x Sound		F(2, 34) = 0.509, p = 0.606		F(2, 43) = 2.229, p = 0.120

Table 28. Locomotor Run 1 Within-Session			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.045	65	0.000	0.605
Repeated Measures ANOVA		G.G.	
Time		F(6.652, 552.138) = 211.098, p = 0.000	
Time x Stress		F(6.652, 552.138) = 1.302, p = 0.250	
Time x Sound		F(13.305, 552.138) = 2.823, p = 0.001	
Time x Stress x Sound		F(13.305, 552.138) = 3.789, p = 0.000	
Stress		F(1, 83) = 0.66, p = 0.798	
Sound		F(2, 83) = 1.377, p = 0.258	
Stress x Sound		F(2, 83) = 3.380, p = 0.039	

Table 29. Locomotor Run 1 Within-Session (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.015	65	0.000	0.577
No Tumors	0.012	65	0.000	0.543
Repeated Measures ANOVA		With Tumors (G.G.)		Without Tumors (G.G.)
Time		F(6.348, 215.820) = 82.516, p = 0.000		F(5.972, 256.777) = 105.789, p = 0.000
Time x Stress		F(6.348, 215.820) = 1.003, p = 0.427		F(5.972, 256.777) = 0.773, p = 0.592
Time x Sound		F(12.695, 215.820) = 1.916, p = 0.031		F(11.943, 256.777) = 1.837, p = 0.043
Time x Stress x Sound		F(12.695, 215.820) = 2.316, p = 0.007		F(11.943, 256.777) = 2.289, p = 0.009
Stress		F(1, 34) = 1.023, p = 0.319		F(1, 43) = 0.003, p = 0.954
Sound		F(2, 34) = 2.88, p = 0.07		F(2, 43) = 1.237, p = 0.30
Stress x Sound		F(2, 34) = 1.029, p = 0.368		F(2, 43) = 4.769, p = 0.013 (M<S&N)

Table 30. Locomotor Run 2 Within-Session			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.060	65	0.000	0.625
Repeated Measures ANOVA		G.G.	
Time		F(6.879, 570.954) = 263.664, p = 0.000	
Time x Stress		F(6.879, 570.954) = 0.807, p = 0.633	
Time x Sound		F(13.758, 570.954) = 1.461, p = 0.078	
Time x Stress x Sound		F(13.758, 570.954) = 1.043, p = 0.409	
Stress		F(1, 83) = 0.162, p = 0.688	
Sound		F(2, 83) = 2.312, p = 0.105	
Stress x Sound		F(2, 83) = 0.540, p = 0.585	

Table 31. Locomotor Run 2 Within-Session (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.014	65	0.000	0.579
No Tumors	0.024	65	0.000	0.565
Repeated Measures ANOVA		With Tumors (G.G.)		Without Tumors (G.G.)
Time		F(6.370, 216.583) = 100.444, p = 0.000		F(6.217, 267.323) = 134.813, p = 0.000
Time x Stress		F(6.370, 216.583) = 1.604, p = 0.143		F(6.217, 267.323) = 0.682, p = 0.669
Time x Sound		F(12.740, 216.583) = 1.653, p = 0.075		F(12.434, 267.323) = 1.005, p = 0.445
Time x Stress x Sound		F(12.740, 216.583) = 1.005, p = 0.447		F(12.434, 267.323) = 1.140, p = 0.329
Stress		F(1, 34) = 4.270, p = 0.046		F(1, 43) = 0.120, p = 0.731
Sound		F(2, 34) = 6.515, p = 0.004		F(2, 43) = 0.876, p = 0.424
Stress x Sound		F(2, 34) = 4.274, p = 0.022		F(2, 43) = 0.437, p = 0.649

Table 32. Locomotor Run 3 Within-Session			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.097	65	0.000	0.666
Repeated Measures ANOVA		G.G.	
Time		F(7.327, 608.149) = 229.614, p = 0.000	
Time x Stress		F(7.327, 608.149) = 0.936, p = 0.480	
Time x Sound		F(14.654, 608.149) = 0.630, p = 0.847	
Time x Stress x Sound		F(14.654, 608.149) = 1.230, p = 0.245	
Stress		F(1, 83) = 0.202, p = 0.655	
Sound		F(2, 83) = 0.779, p = 0.462	
Stress x Sound		F(2, 83) = 1.407, p = 0.251	

Table 33. Locomotor Run 3 Within-Session (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.017	65	0.000	0.600
No Tumors	0.034	65	0.000	0.572
Repeated Measures ANOVA		With Tumors (G.G.)		Without Tumors (G.G.)
Time		F(6.600, 224.395) = 88.174, p = 0.000		F(6.292, 270.561) = 117.659, p = 0.000
Time x Stress		F(6.600, 224.395) = 0.826, p = 0.561		F(6.292, 270.561) = 0.885, p = 0.510
Time x Sound		F(13.2, 224.395) = 0.771, p = 0.693		F(12.584, 270.561) = 0.972, p = 0.478
Time x Stress x Sound		F(13.2, 224.395) = 0.904, p = 0.551		F(12.584, 270.561) = 1.175, p = 0.299
Stress		F(1, 34) = 0.069, p = 0.794		F(1, 43) = 0.705, p = 0.406
Sound		F(2, 34) = 0.335, p = 0.718		F(2, 43) = 2.219, p = 0.121
Stress x Sound		F(2, 34) = 3.307, p = 0.049		F(2, 43) = 2.413, p = 0.102

Table 34. Locomotor Run 4 Within-Session			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.112	65	0.000	0.705
Repeated Measures ANOVA		G.G.	
Time		F(7.754, 643.558) = 252.599, p = 0.000	
Time x Stress		F(7.754, 643.558) = 2.191, p = 0.028	
Time x Sound		F(15.507, 643.558) = 2.708, p = 0.000	
Time x Stress x Sound		F(15.507, 643.558) = 1.494, p 0.099	
Stress		F(1, 83) = 1.721, p = 0.193	
Sound		F(2, 83) = 1.262, p = 0.288	
Stress x Sound		F(2, 83) = 2.987, p = 0.056	

Table 35. Locomotor Run 4 Within-Session (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.032	65	0.002	0.612
No Tumors	0.046	65	0.000	0.660
Repeated Measures ANOVA		With Tumors (G.G.)		Without Tumors (G.G.)
Time		F(6.731, 228.868) = 101.246, p = 0.000		F(7.263, 312.299) = 122.059, p = 0.000
Time x Stress		F(6.731, 228.868) = 2.364, p = 0.026		F(7.263, 312.299) = 0.841, p = 0.558
Time x Sound		F(13.463, 228.868) = 2.025, p = 0.018		F(14.526, 312.299) = 1.546, p = 0.091
Time x Stress x Sound		F(13.463, 228.868) = 0.976, p = 0.477		F(14.526, 312.299) = 0.994, p = 0.460
Stress		F(1, 34) = 2.295, p = 0.096		F(1, 43) = 0.766, p = 0.386
Sound		F(2, 34) = 2.099, p = 0.138		F(2, 43) = 1.099, p = 0.342
Stress x Sound		F(2, 34) = 7.187, p = 0.002		F(2, 43) = 1.378, p = 0.263

Table 36. Locomotor Run 5 Within-Session			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.186	65	0.000	0.764
Repeated Measures ANOVA		G.G.	
Time		F(8.408, 697.892) = 248.637, p = 0.000	
Time x Stress		F(8.408, 697.892) = 1.234, p = 0.274	
Time x Sound		F(16.817, 697.892) = 0.905, p = 0.567	
Time x Stress x Sound		F(16.817, 697.892) = 1.183, p = 0.273	
Stress		F(1, 83) = 0.125, p = 0.725	
Sound		F(2, 83) = 0.406, p = 0.668	
Stress x Sound		F(2, 83) = 1.572, p = 0.214	

Table 37. Locomotor Run 5 Within-Session (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.031	65	0.002	0.674
No Tumors	0.099	65	0.021	0.741
Repeated Measures ANOVA		With Tumors (G.G.)		Without Tumors (G.G.)
Time		F(7.416, 252.140) = 90.961, p = 0.000		F(8.147, 350.322) = 136.203, p = 0.000
Time x Stress		F(7.416, 252.140) = 1.052, p = 0.397		F(8.147, 350.322) = 1.332, p = 0.225
Time x Sound		F(14.832, 252.140) = 1.060, p = 0.395		F(16.294, 350.322) = 1.112, p = 0.341
Time x Stress x Sound		F(14.832, 252.140) = 0.633, p = 0.818		F(16.294, 350.322) = 1.471, p = 0.106
Stress		F(1, 34) = 0.228, p = 0.636		F(1, 43) = 0.013, p = 0.909
Sound		F(2, 34) = 0.517, p = 0.601		F(2, 43) = 0.147, p = 0.864
Stress x Sound		F(2, 34) = 1.099, p = 0.345		F(2, 43) = 2.556, p = 0.089

Table 38. Locomotor Run 6 Within-Session			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.094	65	0.000	0.701
Repeated Measures ANOVA		G.G.	
Time		F(7.713, 632.501) = 230.977, p = 0.000	
Time x Stress		F(7.713, 632.501) = 1.421, p = 0.187	
Time x Sound		F(15.427, 632.501) = 2.190, p = 0.005	
Time x Stress x Sound		F(15.427, 632.501) = 1.650, p = 0.055	
Stress		F(1, 82) = 2.961, p = 0.089	
Sound		F(2, 82) = 0.085, p = 0.919	
Stress x Sound		F(2, 82) = 0.138, p = 0.871	

Table 39. Locomotor Run 6 Within-Session (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.012	65	0.000	0.566
No Tumors	0.028	65	0.000	0.646
Repeated Measures ANOVA		With Tumors (G.G.)		Without Tumors (G.G.)
Time		F(6.162, 203.343) = 84.45, p = 0.000		F(6.994, 300.758) = 117.923, p = 0.000
Time x Stress		F(6.162, 203.343) = 1.481, p = 0.184		F(6.994, 300.758) = 0.579, p = 0.773
Time x Sound		F(12.324, 203.343) = 1.332, p = 0.201		F(13.989, 300.758) = 1.366, p = 0.168
Time x Stress x Sound		F(12.324, 203.343) = 1.502, p = 0.123		F(13.989, 300.758) = 0.818, p = 0.649
Stress		F(1, 33) = 7.272, p = 0.010		F(1, 43) = 0.023, p = 0.879
Sound		F(2, 33) = 0.649, p = 0.529		F(2, 43) = 0.078, p = 0.925
Stress x Sound		F(2, 33) = 1.865, p = 0.171		F(2, 43) = 1.691, p = 0.196

Table 40. Locomotor Run 7 Within-Session			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.159	65	0.000	0.747
Repeated Measures ANOVA		G.G.	
Time		F(8.215, 673.653) = 244.792, p = 0.000	
Time x Stress		F(8.215, 673.653) = 0.790, p = 0.615	
Time x Sound		F(16.43, 673.653) = 1.63, p = 0.054	
Time x Stress x Sound		F(16.43, 673.653) = 1.478, p = 0.099	
Stress		F(1, 82) = 0.153, p = 0.696	
Sound		F(2, 82) = 0.371, p = 0.691	
Stress x Sound		F(2, 82) = 0.275, p = 0.760	

Table 41. Locomotor Run 7 Within-Session (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.048	65	0.033	0.655
No Tumors	0.084	65	0.007	0.689
Repeated Measures ANOVA		With Tumors (G.G.)		Without Tumors (G.G.)
Time		F(7.209, 237.884) = 89.418, p = 0.000		F(7.575, 325.705) = 125.458, p = 0.000
Time x Stress		F(7.209, 237. 884) = 0.953, p = 0.468		F(7.575, 325.705) = 0.991, p = 0.441
Time x Sound		F(14.417, 237.884) = 1.356, p = 0.174		F(15.149, 325.705) = 1.209, p = 0.262
Time x Stress x Sound		F(14.417, 237.884) = 1.590, p = 0.080		F(15.149, 325.705) = 0.609, p = 0.869
Stress		F(1, 33) = 0.012, p = 0.913		F(1, 43) = 0.028, p = 0.868
Sound		F(2, 33) = 1.380, p = 0.266		F(2, 43) = 0.137, p = 0.872
Stress x Sound		F(2, 33) = 3.445, p = 0.044		F(2, 43) = 4.003, p = 0.025

Table 42. Locomotor Run 8 Within-Session			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.140	65	0.000	0.747
Repeated Measures ANOVA		G.G.	
Time		F(8.215, 665.440) = 239.291, p = 0.000	
Time x Stress		F(8.215, 665.440) = 1.128, p = 0.342	
Time x Sound		F(16.431, 665.440) = 1.371, p = 0.147	
Time x Stress x Sound		F(16.431, 665.440) = 3.691, p = 0.000	
Stress		F(1, 81) = 2.243, p = 0.138	
Sound		F(2, 81) = 0.758, p = 0.472	
Stress x Sound		F(2, 81) = 0.430, p = 0.652	

Table 43. Locomotor Run 8 Within-Session (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.036	65	0.014	0.644
No Tumors	0.061	65	0.001	0.655
Repeated Measures ANOVA		With Tumors (G.G.)		Without Tumors (G.G.)
Time		F(7.085, 226.717) = 87.710, p = 0.000		F(7.209, 309.983) = 119.618, p = 0.000
Time x Stress		F(7.085, 226.717) = 1.056, p = 0.393		F(7.209, 309.983) = 0.631, p = 0.735
Time x Sound		F(14.170, 226.717) = 1.124, p = 0.337		F(14.418, 309.983) = 0.991, p = 0.463
Time x Stress x Sound		F(14.170, 226.717) = 2.525, p = 0.002		F(14.418, 309.983) = 2.001, p = 0.016
Stress		F(1, 32) = 0.182, p = 0.672		F(1, 43) = 0.924, p = 0.342
Sound		F(2, 32) = 1.009, p = 0.376		F(2, 43) = 0.421, p = 0.659
Stress x Sound		F(2, 32) = 3.123, p = 0.058		F(2, 43) = 4.529, p = 0.016

Table 44. Center Time/Total Time Ratio (baseline)	
ANOVA	
Stress	F(1, 83) = 6.511, p = 0.013
Sound	F(2, 83) = 0.472, p = 0.626
Stress x Sound	F(2, 83) = 1.685, p = 0.192

Table 45. Center Time/Total Time Ratio Overall			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.425	27	0.000	0.801
Repeated Measures ANCOVA		G.G.	
Time		F(5.608, 448.630) = 0.474, p = 0.816	
Time x Covariate		F(5.608, 448.630) = 0.667, p = 0.666	
Time x Stress		F(5.608, 448.630) = 0.894, p = 0.494	
Time x Sound		F(11.216, 448.630) = 0.590, p = 0.841	
Time x Stress x Sound		F(11.216, 448.630) = 1.201, p = 0283	
Covariate		F(1, 80) = 23.197, p = 0.000	
Stress		F(1, 80) = 0.561, p = 0.456	
Sound		F(2, 80) = 5.786, p = 0.005	
Stress x Sound		F(2, 80) = 0.853, p = 0.430	

Table 46. Center Time/Total Time Ratio (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.185	27	0.008	0.648
No Tumors	0.318	27	0.016	0.788
Repeated Measures ANCOVA		With Tumors (G.G.)		Without Tumors (sphericity not violated)
Time		F(4.537, 140.647) = 1.786, p = 0.091		F(5.518, 231.752) = 0.707, p = 0.632
Time x Covariate		F(4.537, 140.647) = 1.146, p = 0.338		F(5.518, 231.752) = 1.246, p = 0.286
Time x Stress		F(4.537, 140.647) = 0.466, p = 0.784		F(5.518, 231.752) = 1.746, p = 0.118

Time x Sound	F(9.074, 140.647) = 1.031, p = 0.419	F(11.036, 231.752) = 0.545, p = 0.872
Time x Stress x Sound	F(9.074, 140.647) = 0.883, p = 0.543	F(11.036, 231.752) = 0.981, p = 0.465
Covariate	F(1, 31) = 9.210, p = 0.005	F(1, 42) = 10.114, p = 0.003
Stress	F(1, 31) = 1.389, p = 0.248	F(1, 42) = 0.000, p = 0.996
Sound	F(2, 31) = 3.377, p = 0.047	F(2, 42) = 1.956, p = 0.154
Stress x Sound	F (2, 31) = 0.104, 0.901	F(2, 42) = 1.574, p = 0.219

Table 47. Last Center Time/Total Time Ratio	
ANCOVA	
Covariate	F(1, 80) = 13.631, p = 0.000
Stress	F(1, 80) = 0.092, p = 0.762
Sound	F(2, 80) = 2.551, p = 0.084
Stress x Sound	F(2, 80) = 0.247, p = 0.782

Table 48. Last Center Time/Total Time Ratio (Split File by Tumor)		
ANCOVA	With Tumors	Without Tumors
Covariate	F(1, 31) = 0.973, p = 0.332	F(1, 42) = 8.639, p = 0.005
Stress	F(1, 31) = 0.144, p = 0.707	F(1, 42) = 0.813, p = 0.372
Sound	F(2, 31) = 3.435, p = 0.045	F(2, 42) = 0.139, p = 0.871
Stress x Sound	F(2, 31) = 0.549, p = 0.583	F(2, 42) = 1.673, p = 0.200

Table 49. USV Low (initial)	
ANOVA	
Stress	F(1, 83) = 3.315, p = 0.072
Sound	F(2, 83) = 3.430, p = 0.037
Stress x Sound	F(2, 83) = 3.194, p = 0.046

Table 50. USV Low Overall			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.000	27	0.000	0.179
Repeated Measures ANCOVA		G.G.	
Time		F(1.254, 100.317) = 3.164, p = 0.069	
Time x Covariate		F(1.254, 100.317) = 0.100, p = 0.808	
Time x Stress		F(1.254, 100.317) = 6.962, p = 0.006	
Time x Sound		F(2.508, 100.317) = 7.289, p = 0.000	
Time x Stress x Sound		F(2.508, 100.317) = 8.281, p = 0.000	
Covariate		F(1, 80) = 0.131, p = 0.718	
Stress		F(1, 80) = 4.780, p = 0.032	
Sound		F(2, 80) = 5.716, p = 0.005	
Stress x Sound		F(2, 80) = 1.491, p = 0.231	

Table 51. USV Low (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.000	27	0.000	0.157
No Tumors	0.000	27	0.000	0.219
Repeated Measures ANCOVA		With Tumors (G.G.)		Without Tumors (G.G.)
Time		F(1.102, 34.162) = 0.567, p = 0.473		F(1.535, 64.468) = 2.773, p = 0.083
Time x Covariate		F(1.102, 34.162) = 0.023, p = 0.900		F(1.535, 64.468) = 0.240, p 0.728
Time x Stress		F(1.102, 34.162) = 0.023, p = 0.900		F(1.535, 64.468) = 5.883, p = 0.008
Time x Sound		F(2.204, 34.162) = 2.158, p =0.127		F(3.070, 64.468) = 4.760, p = 0.004
Time x Stress x Sound		F(2.204, 34.162) = 2.149, p = 0.128		F(3.070, 64.468) = 6.442, p = 0.000
Covariate		F(1, 31) = 0.537, p 0.469		F(1, 42) = 0.136, 0.714
Stress		F(1, 31) = 1.932, p = 0.174		F(1, 42) = 1.865, p = 0.179
Sound		F(2, 31) = 0.730, p = 0.49		F(2, 42) = 6.434, p = 0.004

Stress x Sound	F(2, 31) = 0.810, p = 0.454	F(2, 42) = 0.169, p = 0.845

Table 52. Last USV Low	
ANCOVA	
Covariate	F(1, 80) = 0.100, p = 0.753
Stress	F(1, 80) = 0.464, p = 0.498
Sound	F(2, 80) = 0.835, p = 0.438
Stress x Sound	F(2, 80) = 0.919, p = 0.403

Table 53. Last USV Low (Split File by Tumor)		
ANCOVA	With Tumors	Without Tumors
Covariate	F(1, 31) = 0.261, p = 0.613	F(1, 42) = 0.036, p = 0.850
Stress	F(1, 31) = 0.181, p = 0.674	F(1, 42) = 0.874, p = 0.355
Sound	F(2, 31) = 0.268, p = 0.767	F(2, 42) = 2.759, p = 0.075
Stress x Sound	F(2, 31) = 0.266, p = 0.768	F(2, 42) = 5.651, p = 0.007

Table 54. USV High (initial)	
ANOVA	
Stress	F(1, 83) = 0.035, p = 0.851
Sound	F(2, 83) = 0.560, p = 0.573
Stress x Sound	F(2, 83) = 1.438, p = 0.243

Table 55. USV High Overall			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.184	35	0.000	0.654
Repeated Measures ANOVA		G.G.	
Time		F(5.229, 423.548) = 34.501, p = 0.000	
Time x Stress		F(5.229, 423.548) = 2.773, p = 0.016	
Time x Sound		F(10.458, 423.548) = 3.915, p = 0.000	

Time x Stress x Sound	F(10.458, 423.548) = 3.433, p = 0.000
Stress	F(1, 81) = 0.696, p = 0.407
Sound	F(2, 81) = 2.833, p = 0.065
Stress x Sound	F(2, 81) = 0.836, p = 0.437

Table 56. USV High (Split File by Tumor)

Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.37	35	0.000	0.504
No Tumors	0.172	35	0.000	0.664

Repeated Measures ANOVA	With Tumors (G.G.)	Without Tumors (G.G.)
Time	F(4.032, 129.039) = 12.356, p = 0.000	F(5.310, 228.329) = 19.950, p = 0.000
Time x Stress	F(4.032, 129.039) = 0.477, p = 0.776	F(5.310, 228.329) = 2.291, p = 0.043
Time x Sound	F(8.065, 129.039) = 2.523, p = 0.014	F(10.620, 228.329) = 1.726, p = 0.071
Time x Stress x Sound	F(8.065, 129.039) = 1.489, p = 0.167	F(10.620, 228.329) = 2.843, p = 0.002
Stress	F(1, 32) = 0.228, p = 0.595	F(1, 43) = 0.139, p = 0.711
Sound	F(2, 32) = 2.112, p = 0.138	F(2, 43) = 2.625, p = 0.084
Stress x Sound	F(2, 32) = 0.178, p = 0.837	F(2, 43) = 2.419, p = 0.101

Table 57. Last USV High

ANOVA	
Stress	F (1, 81) = 3.552, p = 0.063
Sound	F(2, 81) = 2.122, p = 0.126
Stress x Sound	F(2, 81) = 0.395, p = 0.675

Table 58. Last USV High (Split File by Tumor)		
ANOVA	With Tumors	Without Tumors
Stress	F(1, 32) = 0.354, p = 0.556	F(1, 43) = 2.077, p = 0.157
Sound	F(2, 32) = 1.774, p = 0.186	F(2, 43) = 0.349, p = 0.707
Stress x Sound	F(2, 32) = 0.717, p = 0.496	F(2, 43) = 2.134, p = 0.131

Table 59. Horizontal Activity (baseline)	
ANOVA	
Stress	F(1, 83) = 1.643, p = 0.204
Sound	F(2, 83) = 1.526, p = 0.223
Stress x Sound	F(2, 83) = 2.361, p = 0.101

Table 60. Horizontal Activity Overall			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.311	35	0.000	0.777
Repeated Measures ANOVA		G.G.	
Time		F(6.217, 503.589) = 36.087, p = 0.000	
Time x Stress		F(6.217, 503.589) = 1.59, p = 0.145	
Time x Sound		F(12.434, 503.589) = 2.163, p = 0.011	
Time x Stress x Sound		F(12.434, 503.589) = 2.063, p = 0.017	
Stress		F(1, 81) = 0.80, p = 0.374	
Sound		F(2, 81) = 0.049, p = 0.953	
Stress x Sound		F(2, 81) = 1.007, p = 0.370	

Table 61. Horizontal Activity (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.165	35	0.030	0.712
No Tumors	0.247	35	0.014	0.740

Repeated Measures ANOVA	With Tumors (G.G.)	Without Tumors (G.G.)
Time	F(5.696, 182.285) = 15.65, p = 0.000	F(5.921, 254.614) = 20.469, p = 0.000
Time x Stress	F(5.696, 182.285) = 2.021, p = 0.069	F(5.921, 254.614) = 1.069, p = 0.381
Time x Sound	F(11.393, 182.285) = 2.33, p = 0.010	F(11.843, 254.614) = 1.412, p = 0.162
Time x Stress x Sound	F(11.393, 182.285) = 2.12, p = 0.008	F(11.843, 254.614) = 1.671, p = 0.075
Stress	F(1, 32) = 1.025, p = 0.319	F(1, 43) = 0.072, p = 0.79
Sound	F(2, 32) = 1.411, p = 0.259	F(2, 43) = 0.311, p = 0.734
Stress x Sound	F(2, 32) = 4.108, p = 0.026	F(2, 43) = 3.029, p = 0.059

Table 62. Last Horizontal Activity	
ANOVA	
Stress	F(1, 81) = 2.243, p = 0.138
Sound	F(2, 81) = 0.758, p = 0.472
Stress x Sound	F(2, 81) = 0.430, p = 0.652

Table 63. Last Horizontal Activity (Split File by Tumor)		
ANOVA	With Tumors	Without Tumors
Stress	F(1, 32) = 0.182, p = 0.672	F(1, 43) = 0.924, p = 0.342
Sound	F(2, 32) = 1.009, p = 0.058	F(2, 43) = 0.421, p = 0.659
Stress x Sound	F(2, 32) = 3.123, p = 0.058	F(2, 43) = 4.529, p = 0.016

Table 64. Vertical Activity (baseline)	
ANOVA	
Stress	F(1, 83) = 0.034, p = 0.854
Sound	F(2, 83) = 3.224, p = 0.045
Stress x Sound	F(2, 83) = 1.634, p = 0.201

Table 65. Vertical Activity Overall			
Mauchly's Test of Sphericity			
Mauchly's W	df	Sig.	Greenhouse-Geisser
0.282	27	0.000	0.754
Repeated Measures ANCOVA		G.G.	
Time		F(5.276, 422.058) = 7.163, p = 0.000	
Time x Covariate		F(5.276, 422.058) = 3.205, p = 0.006	
Time x Stress		F(5.276, 422.058) = 1.112, p = 0.354	
Time x Sound		F(10.551, 422.058) = 1.229, p = 0.267	
Time x Stress x Sound		F(10.551, 422.058) = 1.089, p = 0.369	
Covariate		F(1, 81) = 38.933, p = 0.000	
Stress		F(1, 81) = 1.661, p = 0.201	
Sound		F(2, 81) = 2.848, p = 0.064	
Stress x Sound		F(2, 81) = 0.248, p = 0.781	

Table 66. Vertical Activity (Split File by Tumor)				
Mauchly's Test of Sphericity				
	Mauchly's W	df	Sig.	Greenhouse-Geisser
Tumors	0.215	27	0.023	0.667
No Tumors	0.160	27	0.000	0.694
Repeated Measures ANCOVA		With Tumors (G.G.)		Without Tumors (G.G.)
Time		F(4.670, 144.767) = 2.785, p = 0.022		F(4.857, 204.006) = 4.111, p = 0.002
Time x Covariate		F(4.670, 144.767) = 1.348, p = 0.250		F(4.857, 204.006) = 3.931, p = 0.002
Time x Stress		F(4.670, 144.767) = 0.856, p = 0.506		F(4.857, 204.006) = 0.632, p = 0.671
Time x Sound		F(9.340, 144.767) = 1.432, p = 0.177		F(9.715, 204.006) = 1.340, p = 0.213
Time x Stress x Sound		F(9.340, 144.767) = 1.424, p = 0.180		F(9.715, 204.006) = 1.059, p = 0.395
Covariate		F(1, 31) = 12.123, p = 0.002		F(1, 42) = 22.458, p = 0.000
Stress		F(1, 31) = 3.532, p = 0.070		F(1, 42) = 0.023, p = 0.879

Sound	F(2, 31) = 2.192, p = 0.129	F(2, 42) = 2.147, p = 0.129
Stress x Sound	F(2, 31) = 3.767, p = 0.034	F(2, 42) = 2.488, p = 0.095

Table 67. Last Vertical Activity	
ANCOVA	
Covariate	F (1, 80) = 18.589, p = 0.000
Stress	F(1, 80) = 2.535, p = 0.115
Sound	F(2, 80) = 3.810, p = 0.026
Stress x Sound	F(2, 80) = 0.222, p = 0.801

Table 68. Last Vertical Activity (Split File by Tumor)		
ANCOVA	With Tumors	Without Tumors
Covariate	F(1, 31) = 3.684, p = 0.064	F(1, 42) = 17.684, p = 0.000
Stress	F(1, 31) = 0.500, p = 0.485	F(1, 42) = 1.045, p = 0.313
Sound	F(2, 31) = 0.710, p = 0.499	F(2, 42) = 2.062, p = 0.142
Stress x Sound	F(2, 31) = 0.856, p = 0.435	F(2, 42) = 2.716, p = 0.078

APPENDIX E – Within-session Activity

(Write-up of all significant findings)

Within-Session Activity (Simple Learning). In the baseline within-session measure there is a significant main effect of time where horizontal activity decreased over time ($F [5.965, 495.063] = 170.775$, $p < 0.001$). There also was a significant time by stress by sound interaction, but there was no clear pattern that emerged ($F [11.929, 495.063] = 2.606$, $p < 0.01$).

A split-file by tumor status was performed. In animals without tumors there was a significant main effect for time where activity decreased over time ($F [5.598, 240.734] = 75.803$, $p < 0.001$). In animals with tumors there also was a significant main effect for time where activity decreased over time ($F [5.510, 187.340] = 75.857$, $p < 0.001$). In animals with tumors there was a significant main effect for sound where the noise condition had greater amounts of activity than did the silence condition, and the silence condition had more activity than did the music condition (noise > silence > music) ($F [2, 34] = 4.841$, $p < 0.05$). At baseline, all animals appeared to habituate to the locomotor arena. In animals with tumors it appears as if the music condition habituated more than the silence condition, and the music and silence conditions habituated more than the noise condition.

In the Run 1 within-session, there was a significant main effect for time where all conditions decreased activity over time ($F [6.652, 552.138] = 211.098$, $p < 0.001$). There also was a significant time by sound interaction where the silence condition decreased activity at a slower rate than did the music and noise

conditions ($F [13.305, 552.138] = 2.823$, $p = 0.001$). There was a significant stress by sound interaction where the non-stressed music condition had more activity than did the stressed music condition; the silence condition had the steepest decrease in activity followed by the noise condition and then the music condition ($F [2, 83] = 3.380$, $p < 0.05$). A significant time by stress by sound interaction was found but no clear pattern emerged ($F [13.305, 552.138] = 3.789$, $p < 0.001$).

A split-file by tumor status was performed. In rats with tumors there was a significant main effect for time where within-session activity decreased over time ($F [6.348, 215.820] = 82.516$, $p < 0.001$). There also was a significant time by sound interaction ($F [12.695, 215.820] = 1.916$, $p < 0.05$) and a significant time by stress by sound interaction ($F [12.695, 215.820] = 2.316$, $p < 0.01$), but no clear pattern emerged.

There was a significant main effect for time in animals without tumors, where activity decreased over time in all conditions ($F [5.972, 256.777] = 105.789$, $p < 0.001$). There was a significant stress by sound interaction where the non-stressed music condition had more activity than did the stressed music condition, and the stressed silence condition had more activity than did the non-stress silence condition ($F [2, 43] = 4.769$, $p < 0.05$). There also was a significant time by sound interaction ($F [11.943, 256.777] = 1.837$, $p < 0.05$) and a significant time by stress by sound interaction ($F [11.943, 256.777] = 2.289$, $p < 0.01$), but no clear patterns emerged.

At Run 1, all animals appeared to habituate to the locomotor arena. The silence condition appeared to habituate slower than the music and noise conditions. The non-stressed music condition had a steeper learning curve than did the stressed music condition; however, this effect may be due to the animals without tumors. In the non-stressed condition, the silence condition had the steepest learning curve, followed by the noise condition, and then the music condition.

At Run 2 there was a significant main effect for time where all animals decreased within-session activity over time ($F [6.879, 570.954] = 263.664$, $p < 0.001$). A split-file was performed based on tumor status. In animals without tumors there was only a significant main effect for time where all animals decreased activity over time ($F [6.217, 267.323] = 134.813$, $p < 0.001$).

In animals with tumors there was a significant main effect for time where all animals decreased activity over time ($F [6.370, 216.583] = 100.444$, $p < 0.001$). There was a significant main effect for stress where non-stressed animals had greater activity than did stressed animals ($F [1, 34] = 4.270$, $p < 0.05$). There was a significant main effect for sound where the noise condition had greater activity than did the music condition, and the noise and music conditions had greater activity than did the silence condition ($F [2, 34] = 6.515$, $p < 0.01$). There also was a significant stress by sound interaction where the non-stressed noise condition had greater activity than did the stressed noise condition ($F [2, 34] = 4.274$, $p < 0.05$).

At Run 2, all animals habituated to the locomotor arena over time. In animals with tumors, the stressed animals had a steeper decline than did the non-stressed and this effect is especially apparent in animals in the noise condition. Further, it appears as if the silence condition had the steepest learning curve, followed by the music condition, and then finally the noise condition.

At Run 3 there was a significant main effect for time where all animals decreased within-session activity over time ($F [7.327, 608.149] = 229.614$, $p < 0.001$). A split-file was performed based on tumor status. In animals without tumors, there was only a significant main effect for time where animals decreased activity over time in all conditions ($F [6.292, 270.561] = 117.659$, $p < 0.001$).

In animals with tumors, there also was a significant main effect for time where all animals decreased activity over time ($F [6.600, 224.395] = 88.174$, $p < 0.001$). There was a significant stress by sound interaction where the non-stressed music condition had less activity than did the stressed music condition, and the stressed noise condition had more activity than did the non-stressed noise condition ($F [2, 34] = 3.307$, $p < 0.05$).

At Run 3, all animals habituated to the locomotor arena over time. In animals with tumors, there was a stress by sound interaction. The non-stressed music condition had a steeper decline in activity than did the stressed music condition, but the stressed noise condition had a steeper decline in activity than did the non-stressed noise condition.

At Run 4 there was a significant main effect for time where within-session activity declined over time in all conditions ($F [7.754, 643.558] = 252.599$, $p < 0.001$). There was a significant time by stress interaction where the stressed animals had a faster decrease in activity than did the non-stressed animals ($F [7.754, 643.558] = 2.191$, $p < 0.05$). There also was a significant time by sound interaction where there was variable changes across conditions over time ($F [15.507, 643.558] = 2.708$, $p < 0.001$). A split-file was performed based on tumor status. There was only a significant main effect for time in animals without tumors, where all animals decreased activity over time ($F [7.263, 312.299] = 122.059$, $p < 0.001$).

In animals with tumors, there was a significant main effect for time where activity decreased over time in all conditions ($F [6.731, 228.868] = 101.246$, $p < 0.001$). There was a significant time by stress interaction where stressed animals decreased activity at a faster rate than non-stressed animals ($F [6.731, 228.868] = 2.364$, $p < 0.05$). There was a significant time by sound interaction where sound conditions had variable responses over time ($F [13.463, 228.868] = 2.025$, $p < 0.05$). There also was a significant stress by sound interaction where the stressed noise condition had lower activity than did the non-stressed noise condition ($F [2, 34] = 7.187$, $p < 0.01$).

At Run 4, all animals habituated to the locomotor arena. It appears as if the stressed animals habituated at a faster rate than the non-stressed animals, especially in the noise condition. These stress effects may be due to the animals with tumors.

At Run 5 there was a significant main effect for time where there was a decrease in within-session activity over time in all conditions ($F [8.408, 697.892] = 248.637$, $p < 0.001$). A split-file was performed based on tumor status. The only significant effect found was for time in both animals with tumors ($F [7.416, 252.140] = 90.961$, $p < 0.001$) and animals without tumors ($F [8.147, 350.322] = 136.203$, $p < 0.001$). All animals habituated to the locomotor arena over the session.

At Run 6 there was a significant main effect for time where there was a decrease in within-session activity over time ($F [7.713, 632.501] = 230.977$, $p < 0.001$). There was a significant time by sound interaction where animals in the sound conditions had variable responses over time during the session ($F [15.427, 632.501] = 2.190$, $p < 0.01$). A split-file was performed by tumor status. There was only a significant main effect for time in animals without tumors, where activity decreased over time in all conditions ($F [6.994, 300.758] = 117.923$, $p < 0.001$). In animals with tumors, there was also the same effect for time ($F [6.162, 203.343] = 84.45$, $p < 0.001$). There also was a significant stress effect in animals with tumors, where the stressed animals had lower activity than the non-stressed animals ($F [1, 33] = 7.272$, $p = 0.01$).

At Run 6, all animals habituated to the locomotor arena during the session. In animals with tumors, animals exposed to stress had a steeper decline in activity than did the animals that were not exposed to stress.

At Run 7 there was a significant main effect for time where there was a decrease in within-session activity over time ($F [8.215, 673.653] = 244.792$,

$p < 0.001$). A split-file was performed based on tumor status. In animals with tumors, there was a significant main effect for time where all animals decreased activity over the session ($F [7.209, 237.884] = 89.418$, $p < 0.001$). There was a significant stress by sound interaction where the non-stressed music condition had lower activity than did the stressed music condition, but the stressed noise condition had lower activity than did the non-stressed noise condition ($F [2, 33] = 3.445$, $p < 0.05$).

In animals without tumors there was a significant main effect for time where all animals decreased activity over time ($F [7.575, 325.705] = 125.458$, $p < 0.001$). There was a significant stress by sound interaction where in animals in the music condition, animals exposed to stress had lower activity than animals that were not exposed to stress ($F [2, 43] = 4.003$, $p < 0.05$).

At Run 7, all animals habituated to the locomotor arena during the session. In animals with tumors, the non-stressed music condition had a steeper decline than did the stressed music condition, but in the noise condition the opposite occurred, animals exposed to stress had a steeper decline than did animals not exposed to stress. In animals without tumors, stressed animals had a steeper decline in activity than non-stressed animals in the music condition, which is opposite of what occurred in the music condition for animals with tumors.

At Run 8 there was a significant main effect for time where within-session activity declined over time in all conditions ($F [8.215, 665.440] = 239.291$,

$p < 0.001$). There was a significant time by stress by sound interactions, however, no clear pattern emerged as conditions were varied in responses throughout the session ($F [16.431, 665.440] = 3.691$, $p < 0.001$).

A split-file was performed based on tumor status. In animals with tumors, there was a significant main effect for time where activity declined over time ($F [7.085, 226.717] = 87.710$, $p < 0.001$). In animals with tumors, there was a significant time by stress by sound interaction where no clear pattern emerged ($F [14.170, 226.717] = 2.525$, $p < 0.01$). In animals without tumors, there was a significant main effect for time where activity decreased over time in all conditions ($F [7.209, 309.983] = 119.618$, $p < 0.001$). In animals without tumors, there was a significant time by stress by sound interaction where no clear pattern emerged ($F [14.418, 309.983] = 2.001$, $p < 0.05$). In animals without tumors, there was a significant stress by sound interaction where the stressed music condition had lower activity than did the non-stressed music condition ($F [2, 43] = 4.529$, $p < 0.05$).

At Run 8, all animals habituated to the locomotor arena during the session. In animals without tumors there was stress by sound interaction. In the music condition, the animals that were exposed to stress appeared to have a steeper decline in activity than the animals not exposed to stress.